

Activation of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -Peroxides with Relevance to the Catalytic Cycle of Ib RNRs



A thesis presented to the University of Dublin for the degree of
Doctor of Philosophy by

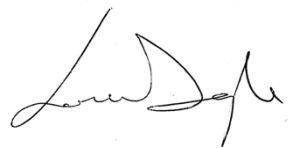
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2023

Author's Declaration

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Lorna Doyle

Summary

The understanding of the catalytic cycle of Ib ribonucleotide reductases (RNRs) has been hindered by the inability to trap the species formed following reaction of superoxide (O_2^-) and the Mn^{II}_2 active site. This unknown intermediate rapidly converted to a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species and oxidised a nearby tyrosine residue to a tyrosyl radical, that was responsible for initiating ribonucleotide reduction. Studying the structure and reactivity of species formed during reaction of biomimetic Mn^{II}_2 complexes with O_2^- may provide some insight into the unknown intermediate in the enzymes catalytic cycle and this was the main objective of this thesis. The main topics of this thesis was the preparation, characterisation, and study of the reactivity of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species as mimics for the unknown intermediate in Ib RNRs.

In chapter 2, upon reaction of O_2^- with $[\text{Mn}^{\text{II}}_2(\text{OCCH}_3)_2(\text{BPMP})](\text{ClO}_4)$ (**1**), where $\text{HBPMP} = 2,6\text{-bis}\{[(\text{bis}(2\text{-pyridylmethyl})\text{amino})\text{methyl}]\text{-4-methylphenol}\}$, the previously published $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ (**2**) was generated. **2** was characterised using UV-vis, EPR, XANES, and ESI-MS techniques. The yield of **2** was optimised upon substitution of the chelating agent 18-crown-6 with 222-Cryptand. **2** was capable of performing aldehyde deformylation of the substrates PPA and CCA. **2** was found to undergo a two-step mechanism during the reaction with aldehydes undergoing an initial reversible nucleophilic attack of the aldehyde by **2** followed by a Criegee rearrangement.

In order to understand the role of the proton in the catalytic cycle of Ib RNRs, the reactivity of **2** towards acids was investigated. Addition of the strong acid HCl to **2** resulted in the formation of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species (**3**) observed by UV-vis spectroscopy and the postulated evolution of H_2O_2 . Attempts to characterise **3** using EPR spectroscopy were unsuccessful due to the formation of “free” Mn^{II} ions that saturated the EPR spectrum. Addition 2 eq. of the weak acid pTsOH to **2** resulted in the generation of a new species (**4**) as evidenced by

the formation of new electronic absorption features ($\lambda_{\text{max}} = 550 \text{ nm}$). New species **4** was further characterised using EPR and X-ray absorption spectroscopies and mass spectrometry and determined to be a new bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. Base reactivity studies with **4** indicated that a proton may reside on the bis(μ -oxo) core of the complex. Using EPR spectroscopy, **4** was found to be capable of the one-electron oxidation of 4-methoxy-2,6-di-*tert*-butylphenol, a functional mimic for the tyrosine residue in Ib RNRs. Further kinetic analysis of the reaction of **4** with O-H bond containing substrates determined that **4** was reacting via a HAT mechanism.

In chapter 4, upon warming of **2** a new species (**5**) was generated as evidenced by the formation of a new broad, featureless electronic absorption feature. Characterisation of **5** via EPR, XAS, and ESI-MS studies determined it to be a new bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. Base studies on complex **5** indicated it contained a “naked” bis(μ -oxo) core, in contrast to the previously characterised **4** in which similar base studies implied the presence of a proton on the bis(μ -oxo) core. **5** was also found capable of the one-electron oxidation of 4-OMe-2,6-DTBP to the corresponding phenoxyl radical species by EPR spectroscopy. In the reaction of **5** with *para*-substituted phenols **5** displayed saturation kinetics indicative of the reversible formation of an intermediate prior to phenol oxidation. We postulated the source of the saturation kinetics was the initial association of the O-H bond of the phenol to the bis(μ -oxo) core which activated the core towards oxidation of the phenol. We speculated that without this initial association step the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ was not reactive enough to oxidise O-H bonds. This was consistent with the observation of linear mechanism in the oxidation of O-H bonds by **4**, which we postulated contained a proton on the bis(μ -oxo) core. This proton increased the electrophilicity of **4** and negated the requirement for the association step required for activation. Further kinetic analysis of the reaction of **5** with O-H bond

containing substrates determined **5** was reacting via HAT mechanism with O-H bond substrates.

Sc(OTf)₃ was added to **2** to investigate the effect in utilising Lewis acids sources as a means to activate the Mn^{II}Mn^{III}-peroxide. Addition of 1 eq. of Sc(OTf)₃ to **2** generated a new species **6** with electronic absorption features at $\lambda_{\text{max}} = 410$ and 550 nm. EPR, XANES, and ESI-MS analysis of **6** determined it to be a new bis(μ -oxo)Mn^{III}Mn^{IV} species. Further EPR analysis found that **6** was capable of the one-electron oxidation of 4-OMe-2,6-DTBP to the corresponding phenoxyl radical. Kinetic analysis of the reaction of **6** with 4-OMe-TEMPO-H established that **6** reacted via a linear mechanism. Interestingly, comparison of the k_I values calculated for the reaction of **4** and **6** with 4-OMe-TEMPO-H under the same conditions revealed that **6** reacted four-times faster than **4**. We postulated that the source of the increased rate was due to a Sc³⁺ ion associated with the core of **6**. As Sc³⁺ was a stronger Lewis acid than H⁺ its presence in **6** resulted in a more electrophilic core and greater reactivity compared to complex **4**. This also provided further weight to the argument that the source of the saturation kinetics of **5** is due to the “naked” bis(μ -oxo) core.

Finally, in chapter 6 a new Mn^{II}₂ complex ([Mn^{II}₂(TPDP)(O₂CC₆H₅)₂](BPh₄) = **7**) was synthesised and characterised using XRD, IR, NMR, XANES, and mass spectrometry techniques and was found to contain two Mn^{II}₂ centres ligated by TPDP and two benzoic acid moieties. Reaction of **7** with O₂⁻ resulted in the formation of a new species (**8**) with electronic absorption features very similar to that of **2** at $\lambda_{\text{max}} = 450$ and 590 nm. **8** was characterised using EPR, XAS, Raman, and mass spectrometry techniques and assigned as a new Mn^{II}Mn^{III}-peroxide species. **8** was, to the best of our knowledge, only the third of its kind to be characterised in the literature. The reactivity of **8** was explored and the Mn^{II}Mn^{III}-peroxide did not react with O-H bonds determined to be poor electrophile. Addition of 2 eq. of pTsOH to **8** resulted in the formation of a new Mn^{II}Mn^{III}-hydroperoxo species (**9**) as

evidenced by UV-vis, EPR, and XANES methods. **9** was found to be capable of the oxidation of the O-H bond of TEMPO-H via EPR spectroscopy. This was an interesting result as **8**, the precursor complex to **9**, displayed no reactivity with the same substrate under the same conditions. Despite this, the reaction of **9** and TEMPO-H was sluggish and afforded only modest yields of the TEMPO $\cdot$ radical.

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step of the way. You deserve at least a masters with how much help you gave me during this thesis.

Abbreviations

18-crown-6	1,4,7,10,13,16-hexaoxacyclooctadecane
222-Cryptand	4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane
<i>A. urinae</i>	<i>Aerococcus urinae</i>
A_{av}	average hyperfine coupling constant
ABNO-H	9-azabicyclo[3.3.1]nonane-N-hydroxide
Asp	aspartate
<i>B. anthracis</i>	<i>Bacillus anthracis</i>
BDE	bond dissociation Energy
BDFE	bond dissociation Free Energy
BEP	Bell-Evans-Polyani
bpy	bipyridine
<i>C. ammoniagenes</i>	<i>Corynebactirum ammoniagenes</i>
CCA	cyclohexanecarboxaldehyde
CPET	concerted proton coupled electron transfer
Cyp	cytochrome
Cys	cysteine
DMF	N,N-dimethylformamide
DNA	deoxyribonucleic acid
DOPA·	3,4-dihydroxyphenylalanine
DPH	diphenylhydrazine
DTBP	di- <i>tert</i> -butylphenol
DTBQ	2,6-di- <i>tert</i> -butyl-1,4-benzoquinone
<i>E. Coli</i>	<i>Escherichia coli</i>
ENDOR	electron-nuclear double resonance spectroscopy
EPR	electron paramagnetic resonance spectroscopy
ESI-MS	electrospray ionisation mass spectra
ET	electron transfer
Et ₂ O	diethyl ether
EXAFS	extended X-ray absorption fine structure analysis
<i>F. johnsoniae</i>	<i>Flavobacterium johnsoniae</i>
Glu	glutamate
HAT	hydrogen atom transfer
HBPMF	2,6-bis[(bis(2 pyridylmethyl)amino)methyl]-4-methylphenol
His	histidine
IR	infrared spectroscopy
KH	potassium hydride
KIE	kinetic isotope effect
<i>L. blandensis</i>	<i>Leeuwenhoekiella blandensis</i>
L1	N,N'-bis-(2-pyrid-2-ylmethyl)-1,4-diazepane)
L2	N-(6-methylpyrid-2-ylmethyl)-1,4-diazepane
LMCT	ligand to metal charge transfer
<i>M. florum</i>	<i>Mesoplasma florum</i>

<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
MeCN	acetonitrile
Mn-CAT	manganese catalase
Mn-SOD	manganese superoxide dismutase
N-Et-HPTB	N,N,N',N'-tetrakis(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3- diaminopropane
NMR	nuclear magnetic resonance
NrdI	flavodoxin
NrdI _{hq}	flavodoxin hydroquinone
NrdI _{sq}	flavodoxin semiquinone
OEC	oxygen-evolving complex
PCET	proton coupled electron transfer
Phe	phenylalanine
PPA	phenylpropionaldehyde
PT	proton transfer
pTsOH	<i>para</i> -toluenesulfonic acid
RFQ	rapid-freeze quench
RNR	ribonucleotide reductase
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>S. pyogenes</i>	<i>Streptococcus pyogenes</i>
SF UV-vis	stopped-flow ultraviolet-visible spectroscopy
<i>S_T</i>	total electronic spin
TEMPO-H	1-hydroxy-2,2,6,6-tetramethyl-piperidine
THF	tetrahydrofuran
TMC	tetramethylcyclam
TOF	time of flight spectrometer
TPDP	1,3-bis(bis(pyridin-2-ylmethyl)amino)propan-2-ol
Trp	tryptophan
Trp ⁺ •	tryptophan radical cation
TRR	thioredoxin reductase
Tyr	tyrosyl radical
Tyr	tyrosine
VtC XES	valence-to-core X-ray emission spectroscopy
XANES	X-Ray absorption near edge spectroscopy
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction analysis

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Chapter 1: Introduction

1.1 Metals in Biology

Carbon, nitrogen, oxygen, and hydrogen are the essential elements for life.¹ However, all organisms also require a variety of transition metals for function of important biological processes.² These metals are incorporated into enzymes and capable of performing powerful chemical processes in ambient conditions. One such metal is manganese, which is utilised in approximately 6% of all enzymes, including: superoxide dismutase, catalase, and ribonucleotide reductase.³ Non-heme manganese enzymes such as manganese catalase (Mn-Cat) and manganese superoxide dismutase (Mn-SOD) protect against reactive oxygen species hydrogen peroxide (H_2O_2) and superoxide (O_2^-) respectively by catalysing the conversion to oxygen and H_2O_2 .^{4,5} Another enzyme that employs non-heme manganese is ribonucleotide reductase, which is responsible for production of deoxyribonucleotides, the monomers for deoxyribonucleic acid (DNA) synthesis.⁶

1.2 Ribonucleotide Reductase

Ribonucleotide reductase (RNRs) are enzymes that possess the unique ability to catalyse the conversion of ribonucleotides to the corresponding deoxyribonucleotides, providing precursors for DNA synthesis and repair in all organisms.⁷ RNRs represent the initial and rate limiting step in DNA synthesis. This key role makes RNRs an important target for anticancer, antiviral, and antibacterial agents.⁸ All RNRs share a common catalytic mechanism whereby a cysteinyl radical is generated *via* oxidation of a conserved cysteine residue in the active site, which then initiates the ribonucleotide reduction.⁶ The three classes of RNR are distinguished by their method generation of the cysteinyl radical (Figure 1.1).

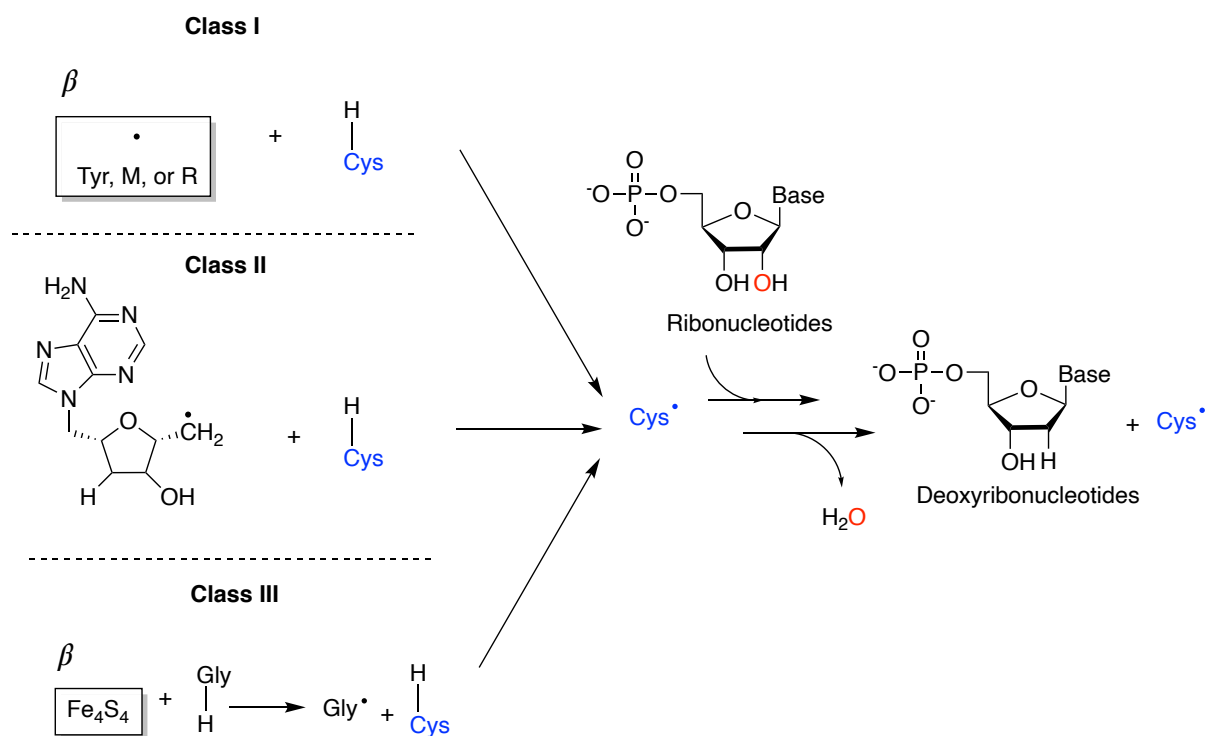


Figure 1.1 : Reduction of ribonucleotides to their corresponding deoxyribonucleotides via the different classes of ribonucleotide reductase.⁹ Source of the radical in the β subunit of class I depends on the subclass and can be a tyrosyl radical (Tyr), a metal based radical (M), or an organic radical (R). Class II utilises a S-adenosylcobalamin cofactor to generate a cysteinyl radical. Class III generates a reactive glycyl radical via a $[\text{Fe}_4\text{-S}_4]$ cluster that is responsible for cysteinyl radical generation.

Class I RNRs are strictly aerobic enzymes. All class I RNRs share the same structural fold that houses two subunits; α and β . The α subunit is the location of the nucleotide reduction and the β subunit houses the radical generating machinery responsible for the reduction. Depending on their subclass class I RNRs can have diiron, dimanganese, mixed metal (Fe and Mn), or no metal in their active site. There are a variety of methods within these subclasses for generation of the cysteinyl radical, including *via* tyrosyl radical, a 3,4-dihydroxyphenylalanine radical (DOPA), or using a high valent metal species (Table 1.1).

Class II and III RNRs harbour similar structures to the α subunit found in Class I, but they utilise different metallocofactors for generation of the radical species. Class II RNRs are found in bacteria and archaea.⁶ This class is oxygen independent and can function in aerobic and anaerobic conditions. Class II RNRs, utilise an S-adenosylcobalamin, a vitamin B₁₂

derivative as a metallocofactor for cysteinyl radical generation (Table 1.1).¹⁰ Class III RNRs can be found in anaerobic bacteria and some bacteriophages.⁶ Class III are inhibited by oxygen and are anaerobic enzymes. Class III RNRs employ a redox active [4Fe-4S] cluster and together with a S-adenylmethionine and reduced flavodoxin generates a stable, but oxygen-sensitive glycyl radical (Table 1.1).¹¹ This glycyl radical is responsible for production of the cysteinyl radical species that initiates nucleotide reduction. Many organisms can possess more than one class of RNR, and the expression of the class depends on the growth conditions.⁶ A prime example of this is the *Escherichia coli* (*E.coli*) bacteria which contains class I and III RNRs, which affords it the ability to survive in aerobic and anaerobic environments.⁹

Table 1.1: The three class of RNRs and their differences.

	Class I	Class II	Class III
Subunit Structure	$\alpha_2\beta_2$	α or α_2	$\alpha_2\beta_2$
Metal Site	Fe ₂ or Mn ₂ or MnFe or No Metal	Deoxyadenosyl- Cobalamin	4Fe-4S, S-adenosylmethionine
Radicals Involved in Turnover	Tyrosyl, DOPA, Cysteinyl	Cysteinyl	Glycyl, Cysteinyl
Oxygen Dependence	Aerobic	Oxygen Independent	Anaerobic
Occurrence	Eukaryotes, Eubacteria Prokaryotes, Bacteriophages, Viruses	Archaeobacteria, Eubacteria	Archaeobacteria, Eubacteria, Bacteriophages

As mentioned previously, all RNR classes share a common catalytic mechanism whereby the metallocofactor is either directly or indirectly involved in the conversion of the conserved cysteine to a cysteinyl radical species in the α subunit (Figure 1.2). The first step of catalysis involves the generation of the cysteinyl radical via oxidation by the metallocofactor specific to the class of RNR.¹² The cysteinyl radical can then initiate the nucleotide reduction via abstraction of the nucleotide's 3' hydrogen atom. A 3'-keto-2'-deoxynucleotide radical is generated upon loss of H₂O. This new radical species is then reduced by the nearby pair of cysteine residues in two proton coupled electron transfer (PCET) steps. The first PCET yields a disulphide radical-anion intermediate and a 3'-keto-2'-deoxyribonucleotide. The following PCET generates a 3'-deoxynucleotide radical and a disulphide.¹² The final catalytic step involves reduction of the 3'-deoxynucleotide to a deoxyribonucleotide and the regeneration of the cysteinyl radical species.

Although the use of a cysteinyl radical to initiate ribonucleotide reduction is conserved in all classes of RNRs, its method of generation is not. In class I and II two active site cysteine residues (shown in green in Figure 1.2) provide the two-electrons for substrate reduction and form a disulphide bridge when they are oxidised. These two cysteines must be re-reduced after every turnover by the exogenous reduction system. In contrast, in class III only one of the cysteine residues is conserved and a formate residue acts as the reductant. It is postulated that the similarity between the mechanisms employed by all RNRs indicated that all three classes are evolutionary related.¹²

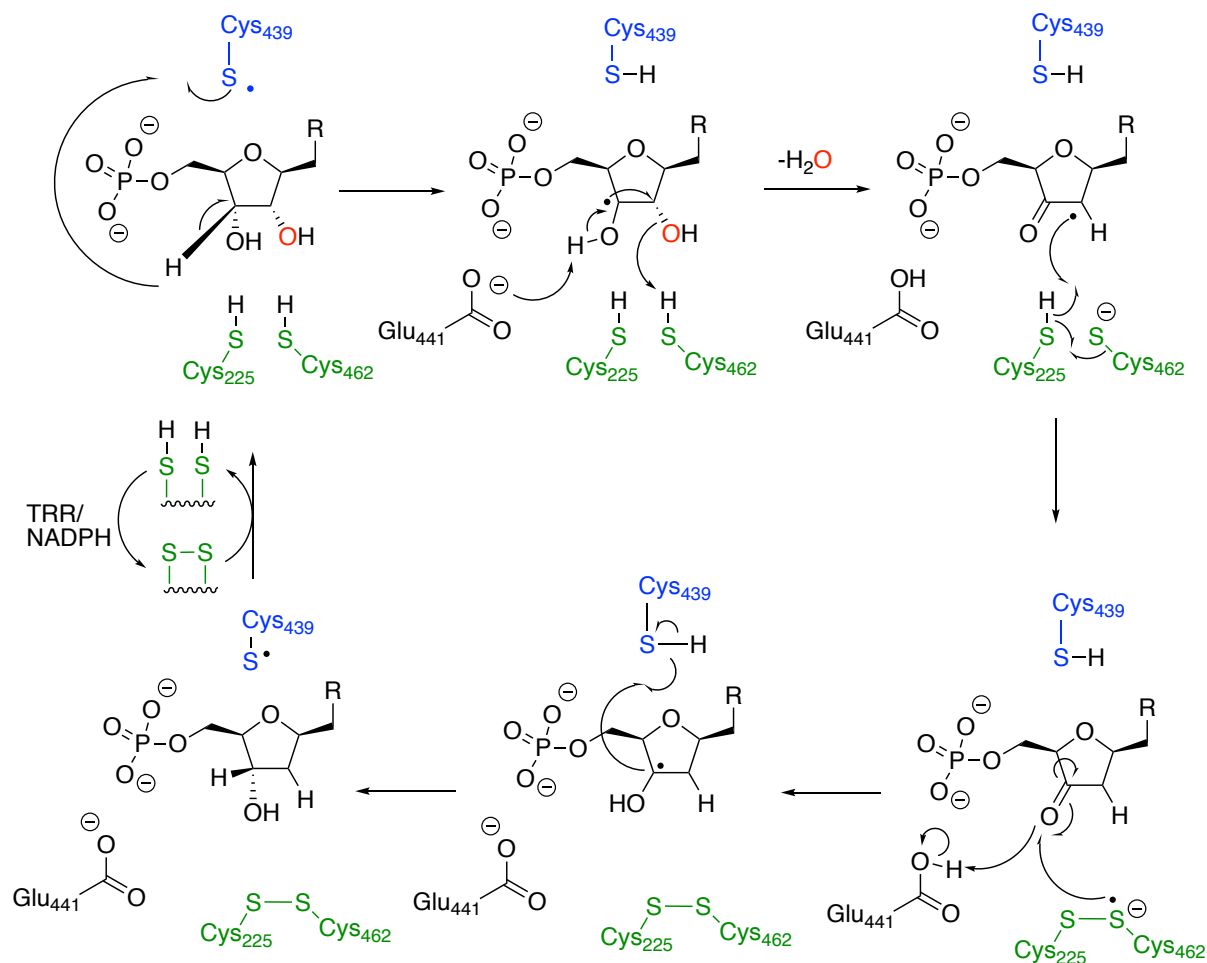


Figure 1.2: The mechanism for ribonucleotide reduction in class I and class II RNRs.¹² The mechanism for class III is similar however it uses formate as external reductant rather than the two cysteine residues (green). Cysteine residues 225 and 462 are reformed via thioredoxin reductase (TRR) and nicotinamide.^{12,13}

1.3 Catalytic cycle of class I RNRs

In the active form of the enzyme, class I RNRs are composed of two homodimers termed $\alpha^2\beta^2$. The first step in radical turnover is postulated to occur via the radical generating cofactor in $\beta 2$, that is triggered by substrate and effector binding in the $\alpha 2$ subunit over 30 Å away.¹⁴ These two subunits must be precisely positioned in order for the radical to move from $\beta 2$, where it is generated, to $\alpha 2$ where substrate reduction occurs.¹⁵ Class I is divided into five subclasses (a, b, c, d, and e) depending on the method of generating a radical in the β subunit.⁶ Following radical generation a series of PCET steps over a distance of approximately 32 Å ensues the delivery of the radical to the α subunit where the cysteine

residue is located (Figure 1.3).¹⁶ Once the cysteinyl radical is generated ribonucleotide reduction is initiated. The details of this radical transfer pathway between subunits have been elucidated via site-specific incorporation of unnatural amino acids into the pathway residues.^{16–18} Through a variety of experimental studies on class I RNRs, the proposed pathway involves $Y_{122}\beta \leftrightarrow [W_{48}\beta] \leftrightarrow Y_{356}\beta \leftrightarrow Y_{731}\alpha \leftrightarrow Y_{730}\alpha \leftrightarrow C_{439}\alpha$ (The involvement of $[W_{48}]$ has not yet been experimentally demonstrated).¹⁹ Each step of the pathway is postulated to involve distinct PCET steps. Binding of the substrate and allosteric effector to α_2 triggers a radical transfer from the $Y_{122}\cdot$ of the Fe^{3+}_2 - $Y_{122}\cdot$ cofactor in the β subunit through three transient Y's across the subunit interface ($356\beta \rightarrow 731\alpha \rightarrow 730\alpha$, Figure 1.3).¹⁹ $Y_{730}\cdot$ is responsible for oxidation of the active site cysteine to a cysteinyl radical. Following ribonucleotide reduction $Y_{122}\cdot$ is regenerated via reverse radical transfer.

two Fe atoms in the active site are coordinated by one aspartate (Asp₈₄), two histidines (His₁₁₈ and His₂₄₁) and three glutamate residues (Glu₁₁₅, Glu₂₀₄, Glu₂₃₈).

Escherichia coli

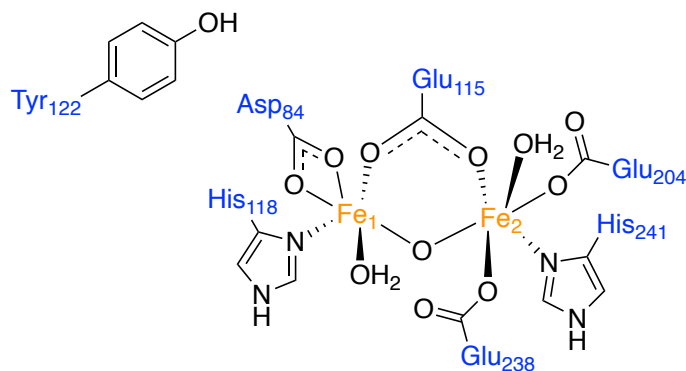


Figure 1.4: Metallocofactor structure found in class Ia RNR *E. coli*.²¹

It was demonstrated that incubation of apo- $\beta 2$ of *E. coli* with Fe^{II} , O_2 , and reductant resulted in the formation of a $\text{Fe}^{\text{III}}\text{Fe}^{\text{III}}\text{-Tyr}^\bullet$ cofactor capable of supporting deoxyribonucleotide formation.²² The enzyme activity was found to be proportional to the concentration of $\text{Tyr}^\bullet$ formed. Studies to understand the metallocofactor mechanism have been undergoing since the 1990s and employing a variety of biophysical methods including stopped-flow ultraviolet-visible (SF UV-vis) spectroscopy, rapid-freeze quench (RFQ) electron paramagnetic resonance (EPR), electron-nuclear double resonance (ENDOR), Mössbauer, and X-ray absorption spectroscopy (XAS).^{13,23–25} The cumulation of these studies has resulted in the proposal of the metallocofactor catalytic mechanism as shown in Figure 1.5. It was postulated that upon loading with Fe, the Fe^{II}_2 - $\beta 2$ reacted rapidly with O_2 resulting in the formation of a diferric-peroxide. The diferric peroxide was reduced by a nearby tryptophan (Trp₄₈) to form a Trp radical cation ($\text{Trp}^{\bullet+}$) and a $\text{Fe}^{\text{IV}}\text{Fe}^{\text{III}}$ species, termed intermediate **X**.⁶ $\text{Trp}_{48}^{\bullet+}$ was converted back to Trp₄₈ via an external reductant. Intermediate **X** was responsible for the PCET oxidation of the Tyr₁₂₂ moiety to a tyrosyl

radical (Tyr₁₂₂[•]). Although the stability of the tyrosyl radical is organism dependent, after generation RNRs can undergo many turnovers of conversion of ribonucleotide to deoxyribonucleotides. In this postulated cycle, four electrons are required for the reduction of dioxygen to water. The oxidation of Fe^{II}₂ to Fe^{III}₂ generated two electrons, and oxidation of tyrosine (Tyr₁₂₂) to a tyrosyl radical (Tyr₁₂₂[•]) provided one-electron. The source of the fourth electron is still unknown, possibly sourced from a nearby protein.²⁶

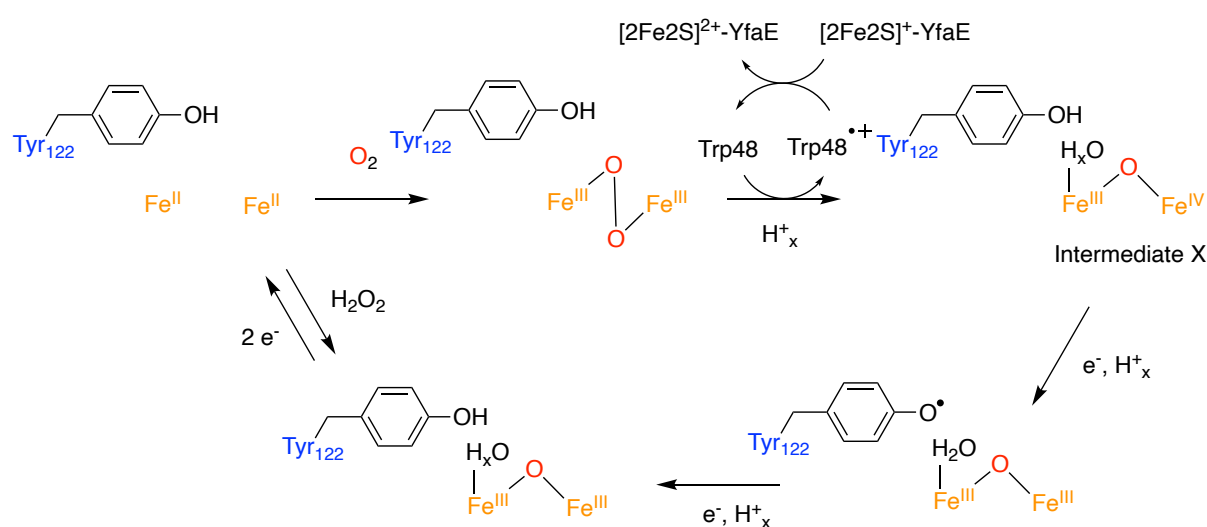


Figure 1.5: Proposed cofactor assembly mechanism of Ia RNRs in *E. coli*.^{6,26}

1.3.2 Ib RNRs

Class Ib RNRs have identical-metal binding residues to class Ia, and for many years there was great controversy over whether Ib RNRs utilised Fe or Mn in the active site. It wasn't until 2010, that Stubbe and co-workers provided definitive evidence that class Ib RNRs utilised a Mn^{III}₂-Tyr[•] cofactor.²⁷ Class Ib RNRs can be found in prokaryotes and such pathogens as *Staphylococcus* (*S. aureus*), *Mycobacterium tuberculosis* (*M. tuberculosis*), *Bacillus anthracis* (*B. anthracis*), *E. coli*, *Corynebacterium ammoniagenes* (*C. ammoniagenes*) and *Bacillus subtilis* (*B. subtilis*).⁶ Interestingly, the two subunits, α 2 and β 2, of classes Ia and Ib are structurally homologous and contain almost identical metal

binding residues, with only small differences in the second coordination sphere differentiating them.²⁸

The crystal structure of *E.Coli* Mn^{II}₂- β 2 revealed two six-coordinate Mn^{II} metal sites (Figure 1.6).²⁹ The two metal centres were bridged by three glutamate residues; Glu₉₈, Glu₁₅₈, and Glu₁₉₂ and displayed a Mn $\cdots$ Mn separation of 3.7 Å. The Mn centre in site one was also coordinated by His₁₀₁, Asp₆₇, and a H₂O molecule and site two ligated by His₁₉₅ and a terminal H₂O molecule.

The crystal structure of *B.subtilis* Mn^{II}₂- β 2 was found to be slightly different from that of *E.coli* Mn^{II}₂- β 2 with only one fully occupied and ordered Mn site (Figure 1.6).³⁰ This crystal structure indicated that Ib RNRs contained diverse coordination geometries. The two Mn sites were found to be further apart in *B.subtilis* at 3.9 Å compared to *E.coli*'s distance of 3.7 Å (Table 1.2). Both Mn centres in *B.subtilis* are bridged by glutamate residues Glu₉₇ and Glu₁₉₈. However, the Mn in site two is coordinatively saturated as it is also ligated by Glu₁₆₄, His₂₀₁, and a terminal H₂O molecule, while the Mn in site one is five-coordinate, with His₁₀₁ and Asp₆₆ in bidentate modes. The difference in coordination number for *B.subtilis* and *E.coli* may be the source of the difference of Mn $\cdots$ Mn separation between the two.

The crystal structure of Mn₂- β 2 was isolated in *C. ammoniagenes* was isolated in 2010 and revealed the shortest Mn $\cdots$ Mn separation of just 3.3 Å (Figure 1.6).³¹ However, it is worth stressing that both Mn centres in *C. ammoniagenes* were in a +3 oxidation state in contrast to the +2 state of the Mn centres in the crystal structures of *E.coli* and *B.subtilis*. *C. ammoniagenes* contained two Mn centres with a oxo/hydroxo bridge near a Tyr residue. The Mn-O_{oxo/hydroxo}-Mn angle was determined to be approximately 120°. The two Mn sites are also bridged by a shared glutamate residue, Glu₁₀₈. The Mn in site one is five coordinate,

ligated by Asp₇₇, His₁₁₁, and a terminal water ligand. The Mn in site two is coordinative saturated with a terminal H₂O, Glu₂₀₂, Glu₁₆₈, His₂₀₅, and the bridging Glu₁₀₈ ligandiagen.

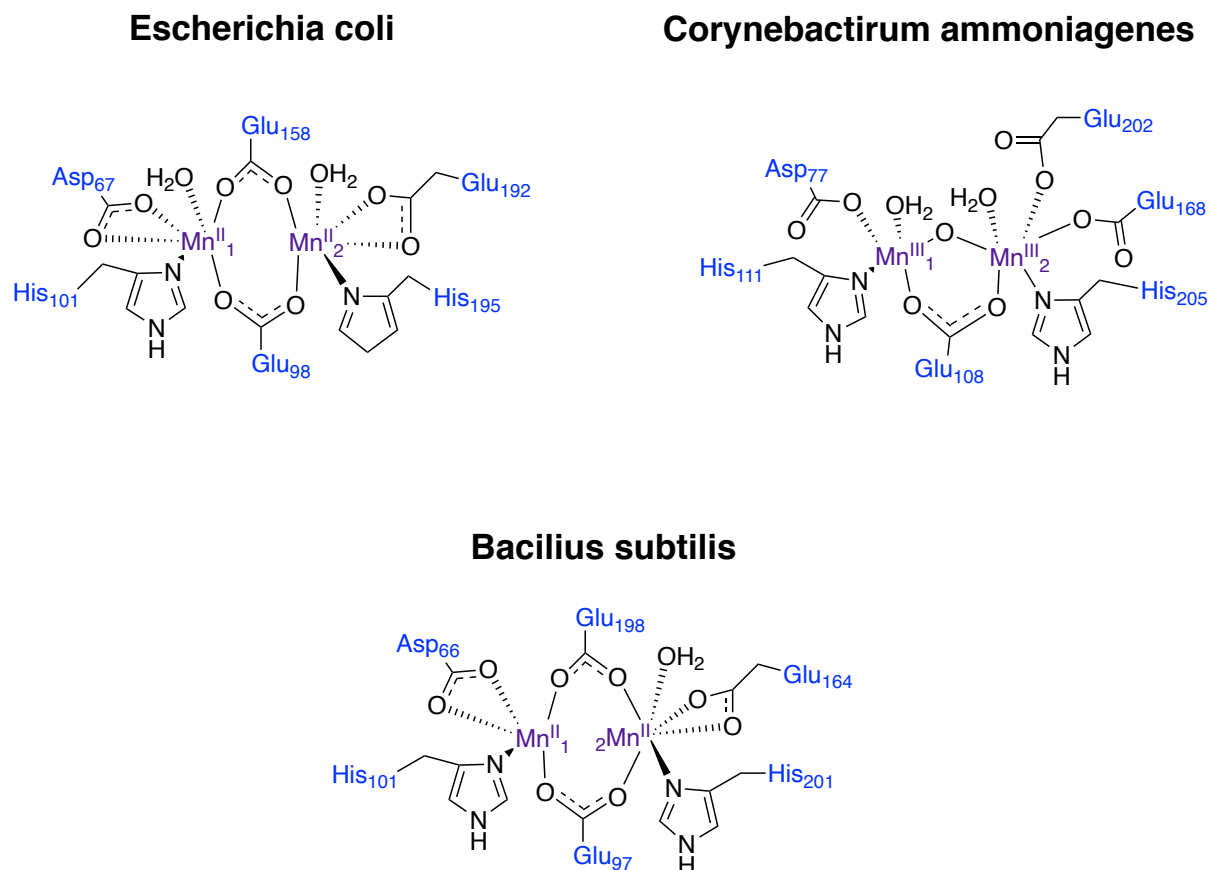


Figure 1.6: Metallocofactors found in class Ib RNRs.^{29,30,32}

Table 1.2. Comparison of the structures of *E. coli*, *B. subtilis*, *C. ammoniagenes* of class Ib RNRs where M = metal.

	<i>E. coli</i> (Mn ^{II} ₂)		<i>B. subtilis</i> (Mn ^{II} ₂)		<i>C. ammoniagenes</i> (Mn ^{III} ₂)	
	Mn1	Mn2	Mn1	Mn2	Mn1	Mn2
Coordination Number	6	6	6	5	6	5
M ... M (Å)	3.7 (± 0.1)		3.9 (± 0.1)		3.3 (± 0.1)	

In the crystal structures of Ib RNRs the Mn metal centres were all five or six coordinated and include solvent water molecule coordination. This was in contrast to the iron Ia

enzymes that contained no solvent ligands and a lower coordination number. This indicated that H₂O dissociation/ligand reorganisation was required in Ib RNRs for activation of the manganese site and hinted at the first differences between Ia and Ib subclasses.³³ Genetic studies revealed the requirement for a flavodoxin protein (NrdI) for enzyme activity *in vivo*, which lead the Stubbe group to investigate if this protein supplied the active oxidant for cofactor activation.³⁴ Stubbe *et al.* monitored the formation and subsequent decay of the semiquinone form of the flavodoxin protein (NrdI_{sq}) from the hydroquinone form (NrdI_{hq}) via UV-vis absorption spectroscopy.³⁵ This indicated molecular oxygen is reduced by the NrdI_{hq} to form superoxide and the NrdI_{hq} was subsequently oxidised to NrdI_{sq}. In the absence of NrdI_{hq}, even with the presence of a variety of oxidants, no deoxyribonucleotides were generated. Therefore, Stubbe and co-workers postulated that NrdI_{hq} reduced O₂ to O₂⁻ and provided the active oxidant in Ib RNRs, making it essential for activity in the enzyme. These studies also revealed the formation and decay of a new feature at $\lambda_{\text{max}} = 340$ nm not attributed to NrdI, or the tyrosyl radical. It was postulated that the $\lambda_{\text{max}} = 340$ nm feature was the species responsible for tyrosyl radical formation. The identity of the 340 nm feature was determined *via* RFQ-EPR spectroscopy and displayed a 16-line EPR signal at 10 K, characteristic of a bis(μ -oxo)Mn^{III}Mn^{IV} species. This lead Stubbe *et al.* to propose the mechanism illustrated in figure 1.7.

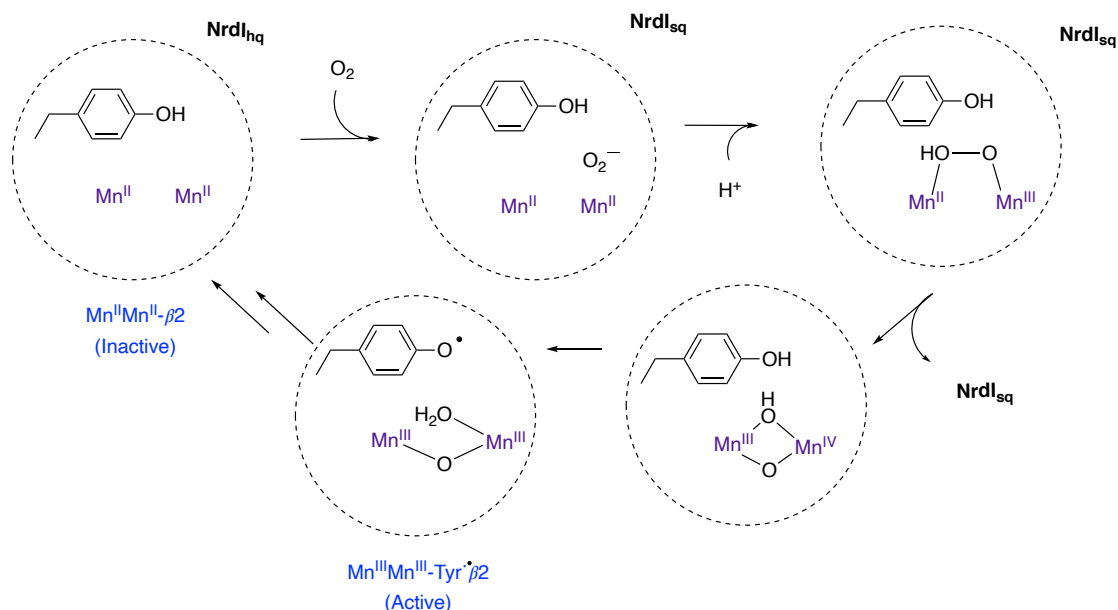


Figure 1.7: Mechanism for class Ib RNRs as proposed by Stubbe *et al.*³⁵

Stubbe *et al.* postulated that molecular oxygen was reduced to superoxide via flavodoxin hydroquinone (NrdI_{hq}). In this process NrdI_{hq} was oxidised to its semiquinone form (NrdI_{sq}). After superoxide was generated via NrdI, the oxidant was channelled to the metal site *via* a hydrophilic tunnel. The crystal structure of *E. coli* NrdI·Mn^{II}Mn^{II}- β 2 complex supported this proposal.²⁹ Once superoxide was delivered to the active site, Stubbe proposed that it was responsible for oxidising the Mn^{II}Mn^{II} cluster, however, the exact mechanism remains unclear.

Crystallographic and biochemical studies suggested that superoxide reacted with the Mn^{II} centre furthest from the tyrosine. From this, Stubbe *et al.* proposed, the formation of a Mn^{II}Mn^{III}-hydroperoxide species which undergoes O-O bond cleavage to form an $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. However, the question remains if superoxide binds in its protonated (HO_2) or the unprotonated (O_2^-) form. O_2^- could react with the Mn^{II}Mn^{II} cluster to generate Mn^{II}Mn^{III}-peroxide species which is then protonated. On the other hand, HO_2 could react to directly to produce a Mn^{II}Mn^{III}-hydroperoxide, similar to azide binding

in hemerythrin, in which hydrozoic acid (HN_3) was proposed to bind to the metal site.³⁵ Although $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ cores display characteristic UV-vis absorption and EPR spectra, no evidence of the formation of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species was found during Stubbe's investigation.^{35–38} It was argued that the features of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species were masked by the strong absorbing NrdI protein in the UV-vis spectrum. If a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species was generated in the reaction, its formation was expected to be fast, analogous to a similar reaction performed in Mn-SOD with rate = $10^9 \text{ M}^{-1} \text{ s}^{-1}$.³⁵ Stubbe *et al.* proposed the reason for their inability to detect a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species was due to a rate-limiting conformation change when O_2^- is delivered to the active site.

Monitoring the decay of the 16-line EPR signal revealed that it was concurrent with the formation of the tyrosyl radical, demonstrating this signal was associated with the species responsible for tyrosine oxidation. The 16-line signal displayed a spectral width of 130 mT, consistent with previously characterised strongly exchange coupled $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ dimers (see section 1.4.5). Moreover, the best fit of the 16-line EPR feature was obtained using two ^{55}Mn ions with effective hyperfine interactions of $[-465 -435 -310]$ and $[230 230 240]$ MHz for the Mn^{III} and Mn^{IV} centres respectively. These values were extremely similar to those determined for the superoxide form of Mn-Cat which contained a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ dimer.³⁹ The $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species formed an order of magnitude slower than NrdI_{sq} production, hinting at the formation of an intermediate species. From this, Stubbe *et al.* suggested that the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ cluster was the active species responsible for tyrosine oxidation to a tyrosyl radical, therefore initiating ribonucleotide reduction in class Ib RNRs. To date, no other spectroscopic evidence exists for this $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species, meaning that no information about the structure and protonation states of this species is known.

The status of the dimanganese cofactor following tyrosine oxidation was investigated using EPR spectroscopy but only a sharp signal at $g = 2.0054$ associated with the tyrosyl radical

was observed.²⁷ From this Stubbe and co-workers surmised that an EPR silent dimanganese product was formed following O-H bond oxidation as radical studies ruled out the possibility of an EPR active mixed valent dimanganese species coupling to the tyrosyl radical.³⁵ Stubbe and co-workers postulated that a $\text{Mn}^{\text{III}}\text{Mn}^{\text{III}}\text{-Y}\cdot$ cofactor was formed following tyrosine oxidation as the final step in the catalytic cycle of Ib RNRs.²⁷

1.3.3 Ic RNRs

In 2007, Bollinger Jr. *et al.* established using a combination of EPR and Mössbauer spectroscopy that subclass Ic RNRs utilised a heterobinuclear Mn/Fe cofactor.⁴⁰ A 1.8 Å resolution crystal structure of the active site of class Ic RNRs was determined in 2013 and revealed the first coordination sphere of Ic was similar to the reduced state structures of Ia and Ib RNRs, however, a phenylalanine (Phe) was found where a Tyr residue would be located in class Ia and b (Figure 1.8).^{29,30,41,42} Phe, in contrast to Tyr, does not produce a radical, therefore, it was postulated in Ic RNRs the Mn/Fe cofactor was responsible for radical production.

In the metallocofactor of Ic RNRs both the Mn and Fe were ligated by one histidine (His_{123} , His_{230}) and four glutamate residues (Glu_{89} , Glu_{227} , Glu_{120} , Glu_{193}). Two of those glutamates (Glu_{120} and Glu_{227}) served to bridge the metal ions. The Mn^{II} centre occupied site one and two exogenous solvent-derived molecules, modelled as water molecules, make the Mn centre six-coordinate. High coordination number Mn centres with bound water have been observed before in the crystal structures of Ib RNRs. There was significant distortion along the $\text{Mn}^{\text{II}}\text{-OH}_2$ (bridging water) axis, imposed by sharing this ligand with the Fe centre and the presence of a phenylalanine Phe_{197} in the outer sphere. The Fe^{II} centre in site two was also six-coordinate and contained a bound water ligand it shared with Mn^{II} . This was unusual as the Fe^{II} centres in class Ia RNRs, typically displayed highly-distorted four and

five-coordinate geometries with no solvent molecules present in the first-coordination sphere.^{29,42,43} Similar distortion was observed along the Fe-H₂O axis for the same reasons outlined for the Mn centre.

Chlamydia trachomatis

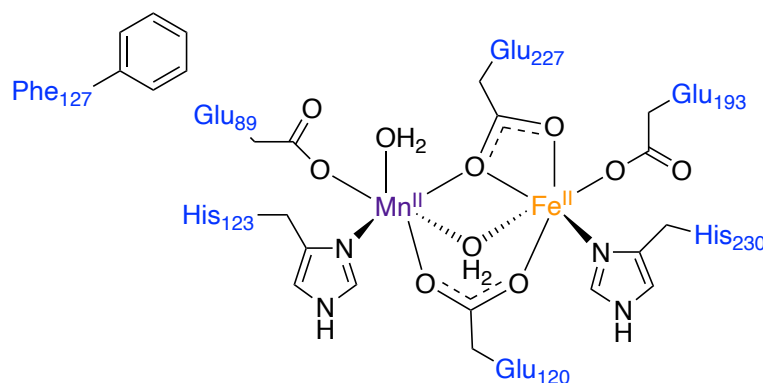


Figure 1.8: Metallocofactor structure in class *Chlamydia trachomatis* Ic RNRs Mn^{II}/Fe^{II}- β .⁴¹

The reaction of the Mn^{II}Fe^{II} resting state with O₂ resulted in the activation of the enzyme indicating that O₂ was the active oxidant for this subclass.⁴⁰ The structure of the active oxidant has not yet been crystallographically characterised, however, a variety of spectroscopic techniques have been employed to understand the intermediates formed during the catalytic cycle of Ic RNRs.^{40,44} Early studies pioneered by Bollinger Jr. *et al.* utilised SF UV-vis, RFQ-EPR, and Mössbauer spectroscopies to assign the oxidation states of the metal centres in the catalytic cycle.^{40,45} Upon reaction of Mn^{II}Fe^{II} with O₂ a Mn^{IV}Fe^{IV} species was formed that contains a $S = \frac{1}{2}$ ground state due to antiferromagnetic coupling between the two metal centres (Figure 1.9).⁴⁵ This intermediate was then slowly reduced to Mn^{III}Fe^{IV}, the active species responsible for radical initiation. It was postulated that the reduction proceeds via a two-step pathway via Trp₅₁ and Tyr₂₂₂.⁴⁶ Tyr₂₂₂ was conserved in the β subunit of Ic RNRs, however, it was not essential for cluster assembly or reactivity.⁴⁷ It was demonstrated that 2 eq. of H₂O₂ can also provide the four oxidising equivalents

required by the $\text{Mn}^{\text{II}}\text{Mn}^{\text{II}}$ resting state to form the high valent $\text{Mn}^{\text{IV}}\text{Fe}^{\text{IV}}$ species.⁴⁸ The $\text{Mn}^{\text{II}}\text{Fe}^{\text{II}}$ state reacted with the first equivalent of H_2O_2 to generate a $\text{Mn}^{\text{III}}\text{Fe}^{\text{III}}$ species which then reacted with the second equivalent of H_2O_2 to produce the $\text{Mn}^{\text{IV}}\text{Fe}^{\text{IV}}$ species. The high-valent $\text{Mn}^{\text{IV}}\text{Fe}^{\text{IV}}$ decayed to form the catalytically active $\text{Mn}^{\text{IV}}\text{Fe}^{\text{III}}$ species.

Much work has been done to identify the metals' oxidation states in the catalytic cycle of Ic RNRs, however, until recently little was known about the structures of the intermediates. One of the breakthroughs in this area was achieved by Pollock *et al.* in 2017. They employed a combination of EXAFS and ENDOR techniques that suggested the lack of strong hyperfine coupling to hydrogen in the $\text{Mn}^{\text{IV}}\text{Fe}^{\text{IV}}$ species indicated it possessed a bis(μ -oxo) core with a terminal OH ligand bound to the Mn^{IV} centre (Figure 1.9).⁴⁴ Pollock *et al.* built upon this discovery by applying two-colour valence-to-core X-ray emission spectroscopy (VtC XES) to the catalytically active $\text{Mn}^{\text{IV}}\text{Fe}^{\text{IV}}$ species to understand its protonation states.⁴⁹ It was determined that the $\text{Mn}^{\text{IV}}\text{Fe}^{\text{III}}$ species possessed a (μ -hydroxo)(μ -oxo) structure (Figure 1.9). This was the first case of the VtC XES technique being applied to an enzyme intermediate.

Many discoveries have been made of the catalytic cycle of Ic RNRs since it was discovered that the metallocofactor contained a mixed metal active site. However, many questions still surround this species, namely the mechanism of formation of the $\text{Mn}^{\text{IV}}\text{Fe}^{\text{IV}}$ species following reaction of $\text{Mn}^{\text{II}}\text{Fe}^{\text{II}}$ with O_2 and the source of the reducing electrons.

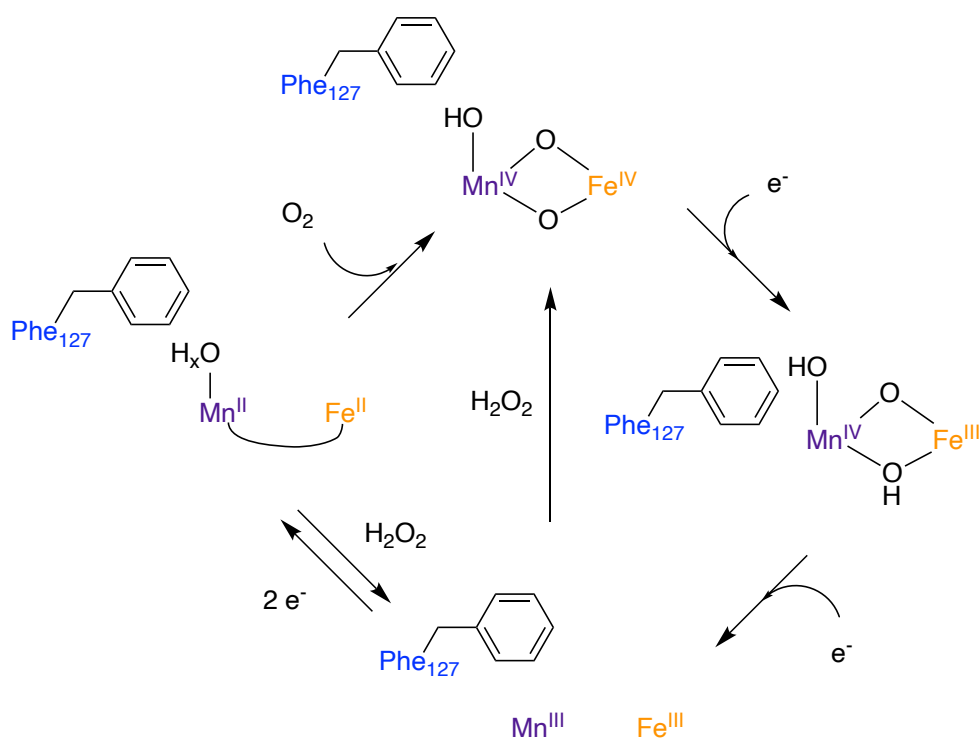


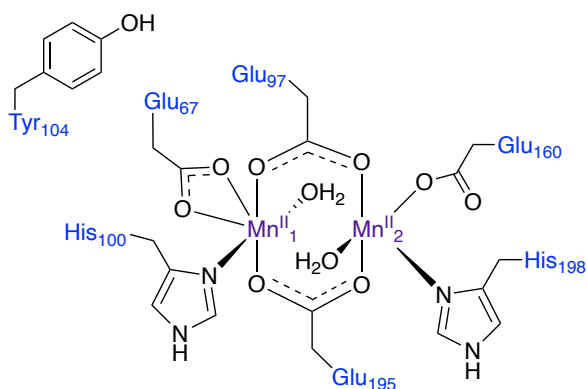
Figure 1.9: Proposed cofactor assembly mechanism of Ic RNRs in *Chlamydia trachomatis*.^{6,41,44,48,49}

1.3.4 Id RNRs

After the discovery of class Ic RNRs, a sequence analysis recognised a correlation between the identity of the residue that corresponds to the Tyr[•] oxidant in classes Ia and Ib and the first metal-ligand residue in the amino acid sequence.³³ In all of the sequences known at the time if a radical generating Tyr residue was located in the β subunit, where the metal cofactor is housed, an Asp was found in the first ligand position (Tyr/Asp). On the other hand, sequences that contained a Phe in place of the Tyr residue, for example Ic RNRs, a Glu was found in the first ligand position (Phe/Glu). In 2019, Boal and co-workers identified over 260 sequences that broke this established correlation by pairing Tyr with Glu.⁵⁰ A sequence similarity analyses suggested that the β subunits containing a Tyr/Glu pairing made up a new cluster, distinct from the previously established subclasses of a, b, and c, and may employ a new cofactor or activation mechanism. Studies of the enzyme *Flavobacterium johnsoniae* (*F. johnsoniae*), that contained the unusual Tyr/Glu

pairing revealed the new subclass and was assigned Id RNRs (Figure 1.10). The isolated crystal structures of *F. johnsoniae* and *Leeuwenhoekiella blandensis* (*L. blandensis*) displayed two fully occupied Mn centres in a +II oxidation state ligated by two His and four Glu, a motif for class I RNRs. Interestingly, the crystal structures showed that the Tyr residue in Id was displaced compared to the radical forming Tyr residue present in subclasses Ia and Ib.^{15,33} In Ia and Ib RNRs, the metal in site 1 shared a hydrogen bond with the phenolic hydroxyl group of the radical harbouring Tyr with an approximate separation of 4-5 Å, possibly facilitating the proton electron transfer steps during formation of the Tyr· radical species. Contrastingly, in the crystal structure of Id RNRs, the Tyr residue was orientated away from the Mn₁ centre and therefore no hydrogen bond was present. This indicated that Id RNRs not use a tyrosyl radical for radical initiation in the reduction of ribonucleotides.

Flavobacterium johnsoniae



Leeuwenhoekiella blandensis

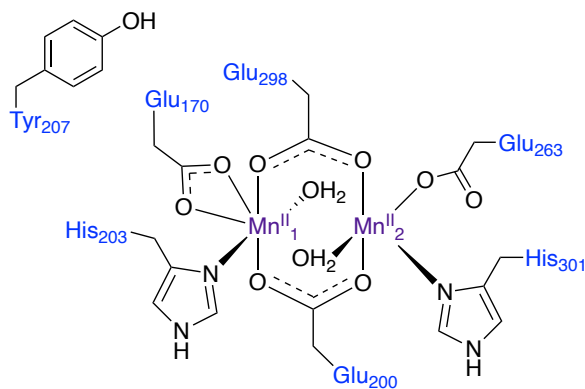


Figure 1.10: Metallocofactor structure *F. johnsoniae* and *L. blandensis* in class Id RNRs Mn^{II}₂-β.^{15,33}

Like class Ib RNRs, this new subclass Id was activated by O₂⁻, however, instead of utilising a NrdI protein to generate O₂⁻, like Ib, the Id metallocofactor was capable of scavenging O₂⁻ from solution.³³ Over 260 Id RNR β subunit sequences have been identified and none of them contained the NrdI protein, further confirming its distinction from the Ib subclass

which required the NrdI for reduction of O_2 to $O_2^{\bullet-}$.³³ UV-vis, EPR, and X-Ray absorption near edge spectroscopy (XANES) studies supported the formation of a $Mn^{III}Mn^{IV}$ species following the reaction of the manganese cluster with superoxide (Figure 1.11). Interestingly, Id RNRs shared a number of similarities with subclass Ib such as; containing a dimanganese cofactor, $O_2^{\bullet-}$ as the activating oxidant, and harbouring a nearby Tyr (Tyr₁₀₄) residue. Yet despite this, no Tyr $\cdot$ radical species was formed during the catalytic cycle of Id RNRs. Instead, the $Mn^{III}Mn^{IV}$ species acted as the radical initiator itself in this subclass.³³ Ib RNRs also formed a $Mn^{III}Mn^{IV}$ species, however only transiently, that quickly decayed to form the $Mn^{III}Mn^{III}$ with concomitant Tyr $\cdot$ radical formation. In Id RNRs, the $Mn^{III}Mn^{IV}$ species can be easily converted back to the Mn^{II}_2 state via three-electron cooperative reduction. This was in contrast to the behaviour observed in the three previously characterised clusters (a, b, and c) as the one-electron reduced clusters ($Fe^{III}Fe^{III}$, $Mn^{III}Mn^{III}$, and $Mn^{III}Fe^{III}$) were found to be inert toward reduction and postulated to require a dedicated reductase for reactivation of the enzyme.⁶

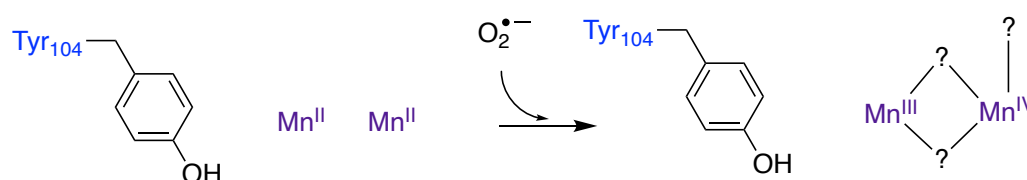


Figure 1.11: Proposed cofactor assembly mechanism for class Id RNRs in *F. johnsoniae*.

1.3.5 Ie RNRs

Class Ie RNRs were independently reported by both Boal *et al.* and Högbom *et al.* in 2018.^{51,52} The sequence of Ie and Ib shared a number of similarities, however, in Ie RNRs the three metal coordinating Glu side chains were not conserved (Figure 1.12). RNR β subunits with this sequence substitution were reported as early as 2008 by Sjöberg and co-

workers, however, in this study no Cys $\cdot$ radical was detected.³⁴ More recently though, experiments using Ie RNR isolated from *Aerococcus urinae* (*A. urinae*) and *Mesoplasma florum* (*M. florum*) were found to be capable of ribonucleotide reduction when α , β , and NrdI were expressed.^{51,52} Over 400 Ie RNR β sequences have been identified using bioinformatic analyses, some of which was the only RNR encoded in the organism's genome.⁵¹

Aerococcus urinae

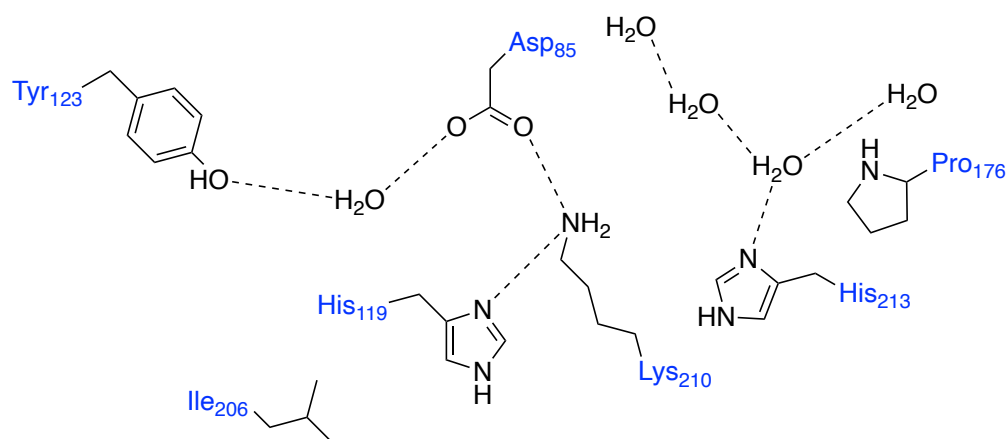


Figure 1.12: Cofactor structure in *A. urinae* Ie RNRs. Hydrogen bonds shown as dashed lines.⁵³

Analysis of *A. urinae*, *M. florum*, and *Streptococcus pyogenes* (*S. pyogenes*) demonstrated that the β subunits of these enzymes contained < 0.2 equivalents of any first-transition row metal. X-ray structures of the active Ie RNRs determined that the β subunit showed no trace of a transition metallocofactor, concluding that Ie RNRs utilised a metal-free mechanism of activation. Further analysis using X-ray crystallography, EPR, and mass spectrometry analysis determined that the radical generating species was a dihydroxyphenylalanine radical (DOPA $\cdot$) that was formed via the posttranslational modification of Tyr₁₂₃, the same site of the Tyr $\cdot$ radical in subclasses Ia/b. Högbom and co-workers proposed that the DOPA $\cdot$ precursor species was post-translationally generated

from hydroxylation of Tyr₁₂₃ via NrdI and O₂ (Figure 1.13). It is still unknown if the DOPA[•] was generated in this single step process. Upon generation of the DOPA[•] radical species in the β subunit it can support multiple ribonucleotide reduction turnovers with the α protein, similar to other I subclasses. If the radical species was quenched, another radical can be regenerated in the β subunit via an oxygen process.

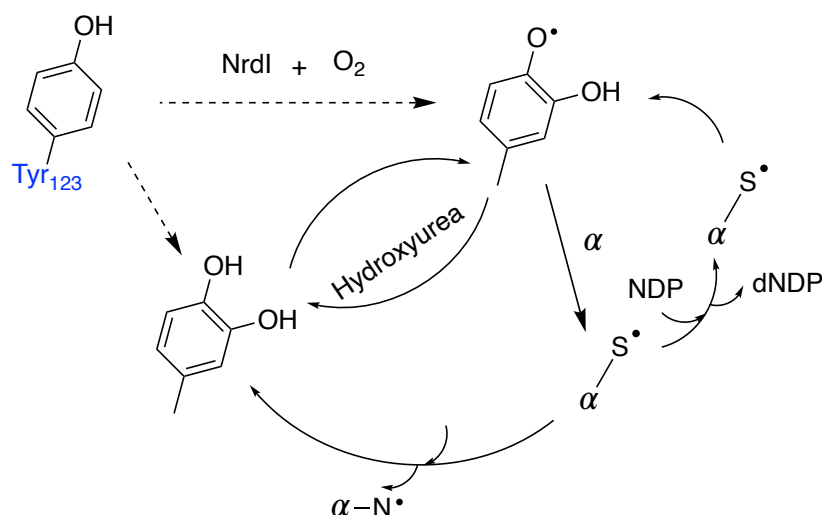


Figure 1.13: Cofactor assembly mechanism of class Ia RNRs.

The rich variety of chemistry employed by all five subclasses of I RNRs is just beginning to be understood. This began over fifty years ago with the discovery of the diiron containing Ia RNRs where Tyr[•] formation was linked to enzyme activity (Table 1.3). Since this time, four more subclasses have been identified; b, c, d, and e. Subclasses c, d, and e utilised a MnFe, Mn^{II}₂ and metal-free cofactors respectively (Table 1.3). Ib RNRs were found to utilise a Mn^{II}₂ metallocofactor and react with O₂^{•-} generated via interaction of O₂ with Nrd_{hq}. However, the intermediate formed following reaction of Mn^{II}₂ and O₂^{•-} has never been trapped and no spectroscopic evidence of this species exists. The unknown intermediate rapidly converted to form a Mn^{III}Mn^{IV} species, confirmed by EPR spectroscopy, although little is known about the structure. This Mn^{III}Mn^{IV} species was responsible for the oxidation

of a nearby tyrosine to generate a Tyr $\cdot$ that initiated ribonucleotide reduction. The ability to understand the mechanism of Ib RNRs has been hindered by the short-lifetimes and high reactivity of the key intermediates. These problems can be subverted by using model complexes of the active sites of Ib RNRs which unlike their enzyme counter parts, can be cooled to cryogenic temperatures, allowing us to trap and analyse their reactive intermediates.⁵⁴ This may allow us to understand the unknown intermediates of Ib RNRs and complement the enzyme mechanistic studies.

Table 1.3: Overview of the subclasses of Ib RNRs

Sub Class	Ia	Ib	Ic	Id	Ie
Metal Site	Fe ₂	Mn ₂	MnFe	Mn ₂	Metal Free
Activating Oxidant	O ₂	O ₂ ⁻	O ₂	O ₂ ⁻	O ₂
Radicals Involved in turnover	Tyr, Cys $\cdot$	Tyr, Cys $\cdot$	Mn ^{IV} Fe ^{III} Cys $\cdot$	Mn ^{III} Mn ^{IV} , Cys $\cdot$	DOPA $\cdot$, Cys $\cdot$

1.4 Model Complexes

Many advances have been made in the field of RNR enzymes including the discovery of new subclasses and breakthroughs in their methods of activation. Despite this, many questions still linger. For example, complete picture of the mechanism of subclass Ib RNRs. One method of gaining a better understanding is creating synthetic models that mimic the enzyme active sites and probing their reactivity. An overview of synthetic manganese complexes that mimic the structures and oxidation states of proposed intermediates in the cycle of class Ib RNRs is provided in the following sections.

1.4.1 Mixed Valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ Complexes

Mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species have been implicated in the mechanism of RNRs and manganese catalases, liver arginase, and Ib RNRs (Section 1.3.2 Figure 1.7).^{35,55,56} Mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes have been synthesised in the literature since the 1980's and characterised using XRD, EPR, and electronic absorption spectroscopies (Figure 1.14).^{57,58} Most $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes were synthesised starting from a Mn^{III} salt, or prepared via oxidation of Mn^{II} precursors.^{37,59–61}

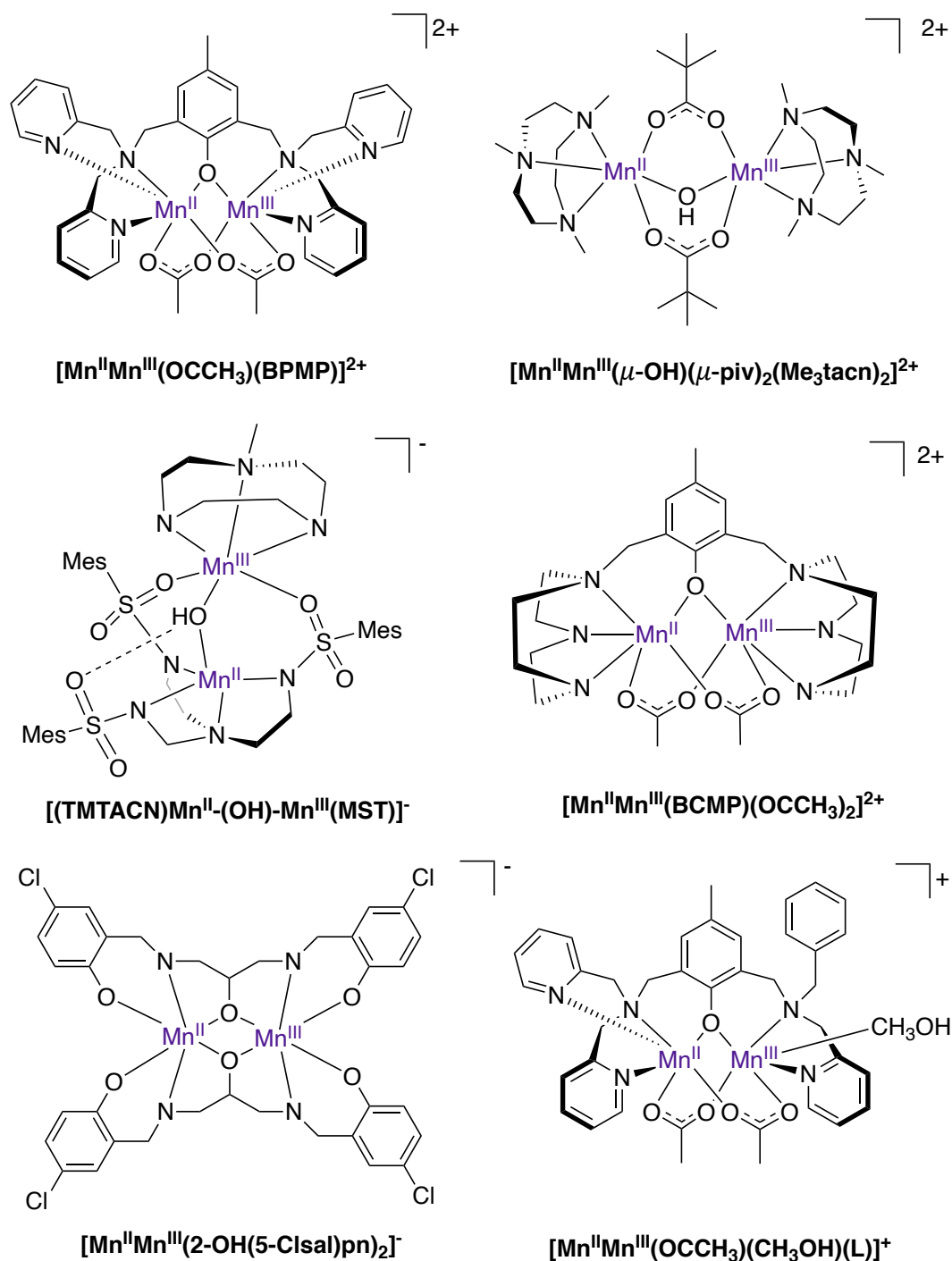


Figure 1.14: Structures of selected mixed valent $Mn^{II}Mn^{III}$ complexes.^{36–38,55,62,63}

Many $Mn^{II}Mn^{III}$ complexes have been crystallographically characterised and many displayed similar Mn ... Mn separations (Table 4). The complex $(TEA)[Mn^{II}Mn^{III}(2-OH)(5-Clal)pn)_2]$. $C_6H_6.CH_3OH$ had one of the shortest Mn ... Mn separations.⁵⁵ This was postulated to be due to the nature of the ligand which contained bridging alkoxy ligands,

possibly drawing the Mn centres closer together. The bond lengths of the related Mn^{II}_2 , Mn^{III}_2 , $\text{Mn}^{\text{III/IV}}_2$ complexes supported by the sal(pn) were 3.3, 3.36, and 3.25 Å respectively, a difference of just 0.11 Å, emphasising that the core structure was not sensitive to changes of oxidation states. Of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes listed in table 1.4, all showed similar Mn ... Mn separations, except for $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\mu\text{-OH})(\mu\text{-piv})_2(\text{Me}_3\text{tacn})_2](\text{ClO}_4)$ which was an outlier with an Mn ... Mn distance of 3.084 Å. It was postulated that the two bridging carboxylate and the μ -hydroxo functionalities of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\mu\text{-OH})(\mu\text{-piv})_2(\text{Me}_3\text{tacn})_2](\text{ClO}_4)$ were the source of the usually short Mn ... Mn distance (Figure 1.14).

Table 1.4: X-ray crystallographic data for selected mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes.

Complex	Bond Length Mn ^{II} ... Mn ^{III} (Å)	Reference
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{BPMP})(\mu\text{-OAc})_2](\text{ClO}_4)_2 \cdot 2\text{H}_2\text{O}$	3.447(1)	37
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{BPMP})(\mu\text{-OAc})_2](\text{ClO}_4)_2 \cdot \text{CH}_2\text{Cl}_2$	3.422(3)	37
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{L})(\text{OAc})_2(\text{H}_2\text{O})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$	3.497	59
$(\text{TEA})[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(2\text{-OH}(5\text{-Cl-sal})\text{pn})_2] \cdot \text{C}_6\text{H}_6 \cdot \text{CH}_3\text{OH}$	3.360(7)	55
$[(\text{TMTACN})\text{Mn}^{\text{II}}(\mu\text{-OH})\text{Mn}^{\text{III}}(\text{MST})](\text{OTf})$	3.472(2)	38
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\mu\text{-OH})(\mu\text{-piv})_2(\text{Me}_3\text{tacn})_2](\text{ClO}_4)$	3.084(3)	64,62
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{BCPMP})(\text{OAc})_2]$	3.473	65

Mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes have also been characterised by UV-Vis absorption spectroscopy. $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes displayed low intensity bands in the range 450-480 nm and 580-630 nm assigned to Mn^{III} *d-d* transitions (Table 5).⁵⁹ $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes may also exhibit bands between 360-400 nm associated with phenoxo- Mn^{III} ligand to metal charge transfer (LMCT).³⁷ $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes have very characteristic EPR spectra. The Mn^{II} ion has a $S_1 = 5/2$ and the Mn^{III} ion a $S_2 = 2$. Both centres coupled together

antiferromagnetically, resulting in a total electronic spin (S_T) of $\frac{1}{2}$.⁶⁶ In continuous-wave EPR the $S_T = \frac{1}{2}$ resulted in a multiline signal centred around $g = 2$. The large number of lines observed in the spectra of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes was due to the non-equivalence of the two manganese centres and the large nuclear spin associated with manganese ($I = 5/2$). This predicted a 36-line signal for isotropic environments ($(2I_1n+1)(2I_2m+1) = 6*6 = 36$). If the g tensor was in an axial environment, then 72 lines would be expected in the EPR spectrum. Moreover, a rhombic g tensor would result in 108 hyperfine lines. However, due to the overlapping of these lines, the number observed in a spectrum varied between 20 and 30. Previously reported EPR spectra of frozen $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes displayed a strong temperature dependence.³⁷ Only at measurement temperatures <15 K were the Mn hyperfine features completely resolved.⁵⁷ When recorded at temperatures >15 K, the signals broadened into the baseline and none of the Mn hyperfine was visible. A broad signal centred at $g = 4$ can also be observed in certain EPR spectra of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes that is assigned to the higher order $S = 3/2$ state.^{37,59,60} All of the EPR measurements of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -complexes have been performed at temperatures <15 K for resolution of the Mn hyperfine features.^{36,37,58,59,67-69} The effective Mn^{55} hyperfine coupling tensors (A_1, A_2) of the coupled $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ system were larger compared to similar antiferromagnetic coupled dinuclear manganese systems e.g. $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ (see Section 1.4.5).⁶⁸ This resulted in broader spectral width for the EPR signals from $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species and made it a distinctive feature of these systems. For example, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{OCCH}_3)(\text{BPMP})]^{2+}$ presented a 29-line signal centred at $g \sim 2$ with a spectral width of 250 mT and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{PhCO}_2)_2(\text{Me}_4\text{alkox})]^{2+}$ displayed a 27-line signal centred at $g = 1.978$ with a spectra width of 180 mT (Table 1.5).^{36,62} Simulation of the EPR spectra of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes are extremely difficult and as a result just a handful have been reported.^{37,66,68}

Mn^{II}Mn^{III} complexes were capable of catalase like reactivity.⁷⁰ For example, [Mn^{II}Mn^{III}(BPBPMP)(OAc)₂](BF₄) demonstrated significant turnover rates in the reaction with H₂O₂ to form O₂ and displayed saturation kinetics that were modelled using the Michaelis-Menten equation.⁷⁰ The neutral complex [Mn^{II}Mn^{III}(BCPMP)(OAc)₂] was found to be a very effective catalyst for the disproportionation of H₂O₂ with a high catalytic turnover number of 7500.⁶⁹

Table 1.5: Electronic absorption features and X-band EPR features for reported $Mn^{II}Mn^{III}$ complexes.

$Mn^{II}Mn^{III}$	Electronic Absorption Features $\lambda_{max}/ nm (\epsilon / M^{-1} cm^{-1})$	EPR Features (No. of Lines, g, width)	Reference
$[(TEA)[Mn^{II}Mn^{III}(2-OH(5-Cl-sal)pn)_2]$	380 (1600)	—	55
$[Mn^{II}Mn^{III}(BPMP)(\mu-OAc)_2](ClO_4)_2 \cdot H_2O$	430 (ND*), 480 (ND), 620 (ND)	29, ~2, 250 mT	37
$[Mn^{II}Mn^{III}(\mu-O)(\mu-piv)(Me_3tacn)_2]$	-	23, 1.966, 180 mT	62
$[Mn^{II}Mn^{III}(PhCO_2)_2(Me_4alkox)]$	-	27, 1.978, 180 mT	62
$[Mn^{II}Mn^{III}(\mu-OAc)_2(BPBMP)]^+$	367(2540), 464 (1870)	-	70
$[Mn^{II}Mn^{III}(\mu-OH)(MeTACN)(MST)](OTf)$	275 (11500), 420 (683), 481 (490), 643 (616), 825 (406)	~19, 2, 180 mT	38
$Mn^{II}Mn^{III}(\mu-OAc)_2BCPMP$	270 (ND), 315 (ND), 450 (ND)	~10, 2, 250 mT	65
$[Mn^{II}Mn^{III}(bttnol)(\mu-O_2C-C_6H_4-NO_2)_2]ClO_4$	212 (100000), 263 (110000), 360 (2591), 479 (1088), 511 (1101), 682 (336)	16-22, 2, ~200 mT	71

*ND = Not Defined

1.4.2 Mononuclear and Dinuclear Mn^{III} -Peroxide Complexes

Mn^{III} -peroxide complexes are of interest due to their implicated reactivity in the cycle of class Ib RNRs, but also in the oxygen evolving complex of photosystem II, and superoxide dismutase.⁷² However, due to the short-lived nature of Mn^{III} -peroxide complexes, no

experimental evidence has been provided for their existence in these cycles.⁷² Studies of mimics of these proposed intermediates can provide insight into the enzymes' mechanism. The first Mn^{III}-peroxide to be crystallographically characterised was [Mn^{III}(O₂)(TPP)]⁻, measured in 1987 (Figure 1.15).⁷³ In the 35 years that have followed only seven Mn^{III}-peroxides have been structurally characterised.^{74–80} The shortage of crystal structures is attributed to Mn^{III}-peroxides' inherent reactivity and short-lifetimes.⁸¹ Nevertheless, many Mn^{III}-peroxides have been generated under cryogenic conditions and characterised via a variety of spectroscopic techniques, mass spectrometry, and computational studies.^{54,76,77,79,82–86}

One of the most common methods of generating Mn^{III}-peroxides is the use of potassium superoxide (KO₂) and a chelating agent to produce soluble superoxide (O₂⁻), which was postulated to react as a one-electron oxidant with the Mn^{II} centre.^{82–85,87} This reaction can be complicated by the superoxide scavenging reactivity of Mn^{II} complexes.⁸⁸ Addition of aqueous H₂O₂ to Mn^{II} complexes provided another method of synthesis of Mn^{III}-peroxides.^{74–77} In some cases, addition of a large excess of H₂O₂ was sufficient for Mn^{III}-peroxide formation, in others, addition of a base such as triethylamine (Et₃N) or anhydrous-H₂O₂-urea was required.^{76,77,79,82,83,85,88–90} Interestingly, the [Mn^{III}(O₂)(TMC)]⁺ species can also be generated from peroxo transfer from related Ni^{III}-O₂ and Co-O₂ complexes supported by 12-TMC and 13-TMC ligands.⁹¹ Some success were achieved via synthesis of Mn^{III}-peroxide complexes via electrochemical generation of superoxide.⁸¹ Formation of the Mn^{III}-peroxide species was monitored via UV-vis and EPR spectroscopies and revealed the use of electrochemically generated superoxide resulted in a more efficient process and minimised the formation of side products. This was of great interest as Mn K-edge X-ray absorption studies of Mn^{III}-peroxides have hindered by their low to moderate yields. The significant increase in yields afforded by the electrochemical generation methods may

make XANES and EXAFS more possible in the future.⁵⁴ Mn^{III} -peroxides can also be generated from the addition of dioxygen to Mn^{II} complexes.^{90,92} For example, treatment of $[\text{Mn}^{\text{II}}(\text{H}_2\text{bupa})]^-$ with dioxygen at room temperature resulted in the formation of $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{H}_3\text{bupa})]^+$ in 50 % yields (Figure 1.15). An increase in the yield and rate of formation of the Mn^{III} -peroxide was observed upon addition of 0.5 equivalents of diphenylhydrazine (DPH) during oxygenation. The related complex, $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{bppa})]$ was also formed via reaction of the low-valent Mn^{II} complex with dioxygen in the presence of H-atom donors such as DPH, indene, and fluorene (Figure 1.15). The increased rate and yields in the presence of H-atom donors indicated that the Mn^{II} centres reacted with O_2 to form a superoxo- Mn^{III} adduct that can abstract a H atom and resulted in a hydroperoxo- Mn^{III} species which undergoes deprotonation or intramolecular proton transfer producing the observed Mn^{III} -peroxide species.

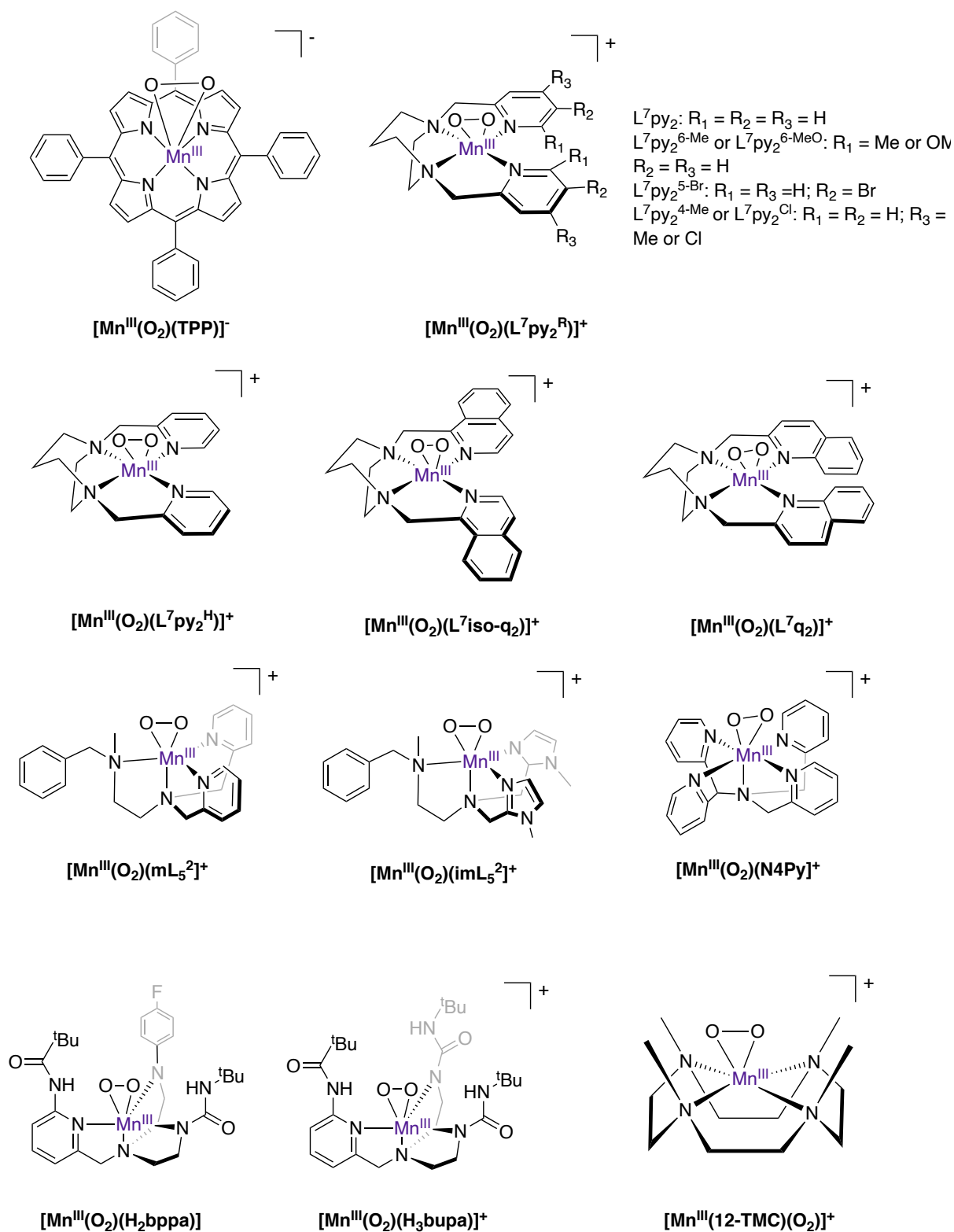


Figure 1.15: Selected structures of Mn^{III} -peroxide complexes. ^{54,79,92,93}

The first peroxo-bridged Mn^{III} complex $[\text{Mn}^{\text{III}}_3(\text{cis-}\mu\text{-1,2-O}_2)(\mu_3\text{-O})(\mu\text{-OCCH}_3)_2(\text{dien})_3]^{3+}$ was isolated by Weatherburn in 1988 upon refluxing $\text{Mn}^{\text{II}}(\text{OCCH}_3)_2$ with dien in methanol and cooling the mixture in air.⁹⁴ Unfortunately, the high symmetry of the complex limited the refinement and resulted in large uncertainties in the metric parameters. More recently, Kovacs and co-workers reported a high-resolution structure of a peroxo-bridged Mn^{III}_2 complex (Figure 1.16).⁹³ $[\text{Mn}^{\text{III}}_2(\text{trans-}\mu\text{-1,2-O}_2)(\text{S}^{\text{Me}_2}\text{N}_4(6\text{-Me-DPEN}))]^{2+}$ was synthesised via addition of dioxygen to the coordinatively unsaturated $[\text{Mn}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(6\text{-Me-DPEN}))]^+$ complex. Low-temperature stop-flow experiments indicated the rapid formation of a $\text{Mn}^{\text{III}}\text{-O}_2$ intermediate, consistent with O_2 binding to an open coordination site. This new $\text{Mn}^{\text{III}}\text{-O}_2$ was found to slowly convert to the peroxo-bridged Mn^{III} species.

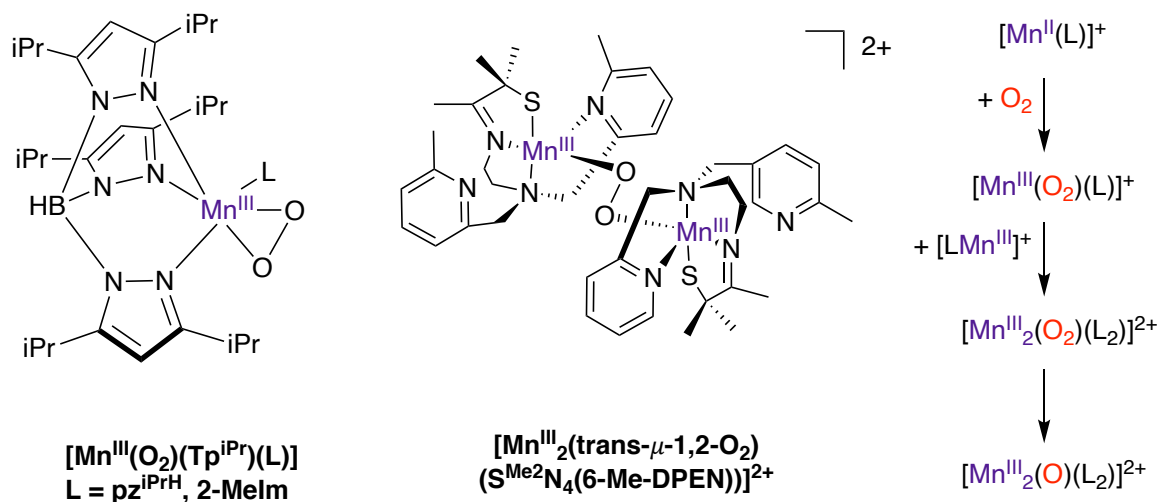


Figure 1.16: Structure of a Mn^{III} -peroxide complex, a binuclear $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, and the scheme to form the binuclear peroxide species (from left to right).^{75,93}

A handful of crystal structures of the Mn^{III} -peroxides supported by a variety of ligands (TPP, Tp^{iPr} , TMC, 13-TMC, 12-TMC) have been reported.^{73–77,79} All of the structures displayed a six-coordinate Mn^{III} centre with a η^2 -peroxide ligand. The Mn-O and O-O bond lengths of these structures ranged from 1.838 to 1.901 Å and 1.402 to 1.43 Å respectively (Table 1.6). The majority of the Mn^{III} -peroxide complexes displayed symmetric Mn-O bonds with bond distances differing by less than 0.015 Å. Of the seven crystal structures of

Mn^{III}-peroxide complexes, two of them are actually isomers of the same complex [Mn^{III}(O₂)(Tp^{iPr})(pz^{iPr2})] (Figure 1.17). The brown form of the isomer was first prepared upon treatment of a bis(μ -hydroxo)Mn^{II}₂ complex (Mn^{II}(HB-3,5-iPr₂pz)₃]₂(OH)₂ with excess H₂O₂ in the presence of 3,5-iPr₂pzH at -20 °C. In toluene, at -20 °C the brown isomer [Mn^{III}(O₂)(Tp^{iPr})(pz^{iPr2})] displayed a shoulder at $\lambda_{\text{nm}} = 561$ nm. Cooling to -78 °C revealed a feature at $\lambda_{\text{nm}} = 583$ nm associated with the blue isomer of the complex. The X-ray crystal structures of the isomers revealed the asymmetry of the Mn-O bonds in the blue form that distinguished it from the brown isomer. This demonstrated the thermochromism displayed by the [Mn^{III}(O₂)(Tp^{iPr})(pz^{iPrH})] complex. The relative short N(pz^{iPr2})-O(peroxo) distance of 2.82(2) and 2.99(2) Å observed in the blue isomer compared to the distances of 3.298(8) and 3.083(3) Å in the brown complex, indicated that intermolecular hydrogen bonds resulted in the asymmetry of the Mn-O₂ moiety.

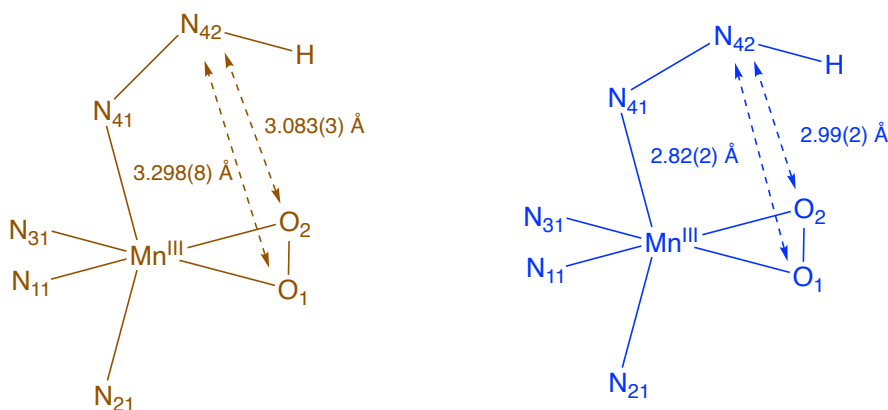


Figure 1.17: Structure of the Mn^{III}-peroxide core of the brown (left) and blue (right) isomers of the [Mn^{III}(O₂)(Tp^{iPr})(pz^{iPr2})] with labelled bond distances.⁷⁴

The coordination geometry of the Mn centres in the Mn^{III}-peroxides can be compared by calculating the τ_5 parameter for each complex. Values of τ_5 range from 0 for square pyramidal to 1 for trigonal bipyramidal geometries.⁵⁴ The Mn^{III}-peroxide supported by the TPP ligand has an almost perfect square pyramidal geometry with $\tau_5 = 0.15$. It was

postulated that the rigid porphyrin structure restricted any differences in the Mn-N bond lengths and N-Mn-N angles. On the other end of the spectrum, the brown and blue isomers of the $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{Tp}^{\text{iPr}})(\text{pz}^{\text{iPrH}})]$ complex both have geometries closest trigonal bipyramidal with τ_5 values of 1.1 and 1.2 respectively. For the TMC derived complexes it was found that as the ring size decreased the Mn centres took on a more square pyramidal geometry. Comparison of the crystal structure of the peroxo-bridged Mn^{III} complex $[\text{Mn}^{\text{III}}_2(\text{trans-}\mu\text{-1,2-O}_2)(\text{S}^{\text{Me}_2}\text{N}_4(6\text{-Me-DPEN}))]^{2+}$ with that of the Mn^{III} -peroxide revealed some interesting differences (Table 1.6). The peroxo-bridged species had an usually long O-O distance and short Mn-O distances of 1.452 and 1.832 Å respectively.⁹³ Compared to bridged-peroxo iron and copper complexes the O-O bond length for $[\text{Mn}^{\text{III}}_2(\text{trans-}\mu\text{-1,2-O}_2)(\text{S}^{\text{Me}_2}\text{N}_4(6\text{-Me-DPEN}))]^{2+}$ falls on the longer end for this class of complex.⁹⁵⁻⁹⁷ This indicated that this bridged-peroxo Mn^{III} was highly activated and ready for O-O bond cleavage.

Table 1.6: Selected bond lengths for Mn^{III}-peroxide and peroxo-bridged Mn^{III} complexes.

Mn ^{III} -peroxide	Mn-O (Å)	O-O (Å)	Reference
[Mn ^{III} (O ₂)(TPP)] ⁺	1.888(4), 1.901(4)	1.421(5)	73
[Mn ^{III} (O ₂)(Tp ^{iPr})(pz ^{iPr2})] (brown)	1.850(6), 1.851(6)	1.428(7)	74
[Mn ^{III} (O ₂)(Tp ^{iPr})(pz ^{iPr2})] (blue)	1.841(9), 1.878(8)	1.43(1)	74
[Mn ^{III} (O ₂)(Tp ^{iPr2})(MeIm)]	1.838(6), 1.872(6)	1.42(1)	75
[Mn ^{III} (O ₂)(TMC)] ⁺	1.884(2), 1.884(2)	1.403(4)	76
[Mn ^{III} (O ₂)(13-TMC)] ⁺	1.855(2), 1.863(2)	1.410(4)	77
[Mn ^{III} (O ₂)(12-TMC)] ⁺ ^a	1.853	1.408	79
[Mn ^{III} ₂ (trans- μ -1,2-O ₂)(S ^{Me2} N ₄ (6-Me-DPEN))] ²⁺	1.832(3)	1.452(5)	93

^a = The peroxo of [Mn^{III}(O₂)(12-TMC)]⁺ is disordered.

The $\nu_{\text{O-O}}$ stretch of Mn-peroxide is Raman active, however, measurement is very difficult as samples typically suffer degradation upon laser irradiation.^{76,87} Due to this, the $\nu_{\text{O-O}}$ stretch has only been measured for five complexes and the $\nu_{\text{Mn-O}}$ has never been observed for a Mn^{III}-peroxide complex (Table 1.7). Of the four η^2 -peroxomanganese adducts for which a $\nu_{\text{O-O}}$ stretch has been reported, all of values fall in a very narrow range between 885-896 cm⁻¹. In contrast, the $\nu_{\text{O-O}}$ band for the bridged-peroxo Mn^{III} complex [Mn^{III}₂(trans- μ -1,2-O₂)(S^{Me2}N₄(6-Me-DPEN))] ²⁺ falls at a much lower energy of 819 cm⁻¹. This was consistent with the long O-O bond length observed for this complex. Interestingly, the $\nu_{\text{Mn-O}}$ band could be observed for the bridged peroxo complex at an energy of 611 cm⁻¹, the only stretch of its kind to have ever been measured.⁹³ The $\nu_{\text{O-O}}$ bands of the Mn^{III}-peroxides

were sensitive to isotopic $^{18}\text{O}_2$ labelling and observed to shift between 47-52 cm^{-1} upon substitution. The $\nu_{\text{Mn-O}}$ band of the peroxo-bridged Mn^{III} was found to shift by 25 cm^{-1} upon $^{16}\text{O}_2/^{18}\text{O}_2$ labelling.

η^2 -peroxomanganese Mn^{III} complexes typically displayed low-intensity electronic absorption features ($\lambda_{\text{max}} = 415\text{-}670\text{ nm}$, $\varepsilon = 60\text{-}490\text{ M}^{-1}\text{ cm}^{-1}$) originating from a combination of peroxide to metal charge transfer and d-d transitions (Table 1.7).^{79,84,86} For example, the well characterised series of Mn^{III} -peroxide complexes supported by TMC ligands; $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{TMC})]^+$, $[\text{Mn}^{\text{III}}(\text{O}_2)(13\text{-TMC})]^+$, and $[\text{Mn}^{\text{III}}(\text{O}_2)(12\text{-TMC})]^+$ all displayed an intense feature at $\lambda_{\text{nm}} = 450\text{ nm}$ and a broader feature between 615 - 630 nm.^{76,77,79} Moreover, this trend was also observed in the Mn^{III} -peroxide species supported by polydentate amine ligands such as $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{L1})]^+$ and $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{L2})]^+$ where L1 = N,N'-bis-(2-pyrid-2-ylmethyl)-1,4-diazepane) and L2 = N-(6-methylpyrid-2-ylmethyl)-1,4-diazepane that displayed two features; an intense band between $\lambda_{\text{nm}} = 440\text{-}450\text{ nm}$ and a less intense one between $\lambda_{\text{nm}} = 556\text{ - }597\text{ nm}$.⁸⁶ In summary, all of the reported Mn^{III} -peroxide complexes have shown very similar electronic absorption features.

Table 1.7: Electronic absorption features and Raman stretches for reported Mn^{III}-peroxoide and peroxo-bridged Mn^{III} complexes

Mn ^{III}	Electronic Absorption Features λ / nm (ϵ / M ⁻¹ cm ⁻¹)	Raman ν_{O-O} , /cm ⁻¹ ($\Delta^{18}\text{O}$ /cm ⁻¹)	Reference
[Mn ^{III} (O ₂)(Tp ^{iPr})(pz ^{iPrH})] (brown)	561 (~50)	892, (51)	74
[Mn ^{III} (O ₂)(Tp ^{iPr})(pz ^{iPrH})] (blue)	583 (~60)	892 (51)	74
[Mn ^{III} (O ₂)(Tp ^{iPr})(Me-Im)]	381 (314), 478 (173)	896	75
[Mn ^{III} (O ₂)(H ₃ bupa)] ⁻	660 (300), 490 (ND)	885 (48)	92
[Mn ^{III} (O ₂)(H ₂ bppa)]	590 (58)	891 (52)	89
[Mn ^{III} (O ₂)(mL ² ₅)] ⁺	585 (335)	-	87,89
[Mn ^{III} (O ₂)(L1)] ⁺	448 (197), 556 (42)	-	86
[Mn ^{III} (O ₂)(L2)] ⁺	440 (175), 597 (41)	-	86
[Mn ^{III} (O ₂)(imL ² ₅)] ⁺	542 (484)	-	83
[Mn ^{III} (O ₂)(N4py)] ⁺	617 (280)	-	83,88
[Mn ^{III} (O ₂)(L ⁷ py ^{H₂})] ²⁺	445 (280), 590 (120)	-	84
[Mn ^{III} (O ₂)(L ⁷ py ^{6-Me₂})] ²⁺	415 (280)	-	84
[Mn ^{III} (O ₂)(L ⁷ py ^{4-Me₂})] ²⁺	445 (260), ~588 (100)	-	85
[Mn ^{III} (O ₂)(L ⁷ py ^{4-Cl₂})] ²⁺	442 (262), 568 (102)	-	82
[Mn ^{III} (O ₂)(L ⁷ iso-q ₂)] ²⁺	446 (291), 587 (112)	-	82
[Mn ^{III} (O ₂)(L ⁷ py ^{6-MeO₂})] ²⁺	416 (250)	-	84
[Mn ^{III} (O ₂)(L ⁷ py ^{5-Br₂})] ²⁺	445 (220)	-	84
[Mn ^{III} (O ₂)(L ⁷ q ₂)] ²⁺	416 (305)	-	82
[Mn ^{III} (O ₂)(L ⁸ py ^{H₂})] ²⁺	464 (280), 599 (120)	-	85
[Mn ^{III} (O ₂)(TMC)] ⁺	453 (490), 630 (120)	-	76
[Mn ^{III} (O ₂)(13-TMC)] ⁺	452 (390), 615 (190)	-	77
[Mn ^{III} (O ₂)(12-TMC)] ⁺	455 (250), 620 (200)	-	79
[Mn ^{III} ₂ (trans- μ -1,2-O ₂)(S ^{Me₂} N ₄ (6-Me-DPEN))] ²⁺	640 (830)	819 (47)	93
[Mn ^{III} (OOH)(TMC)] ⁺²	324 (6400), 384 (8000)	792 (44)	98

In 2014, Nam and co-workers reported the first ever spectroscopically characterised hydroperoxo-Mn^{III} species generated from the reaction of a Mn^{III}-peroxide with an acid (Figure 1.18).⁹⁸ Treatment of [Mn^{III}(O₂)(TMC)]⁺ with 3 equivalents of perchloric acid (HClO₄) resulted in an immediate conversion as evidenced by the formation of the electronic absorption features at $\lambda_{\text{nm}} = 324$ and 384 nm associated with the Mn^{III}-hydroperoxo species [Mn^{III}(OOH)(TMC)]²⁺. Interestingly, it was possible to convert the Mn^{III}-hydroperoxo adduct back to the peroxide species via addition of 3 equivalents of the base triethylamine (NEt₃). This type of interconversion between peroxides and hydroperoxo species via acid-base chemistry was previously observed in Fe^{III}-peroxide and Fe^{III}-hydroperoxo species.^{99,100} Raman studies on the new Mn^{III}-hydroperoxo complex revealed a band at 792 cm⁻¹ that shifted by 44 cm⁻¹ upon ¹⁶O₂/¹⁸O₂ substitution. This value for a O-O stretch of [Mn^{III}(OOH)(TMC)]²⁺ falls approximately 100 cm⁻¹ lower compared to Mn^{III}-peroxide complexes (Table 1.7), but agreed well with the previously characterised Fe^{III}-hydroperoxo complexes (780-840 cm⁻¹).^{101,102}

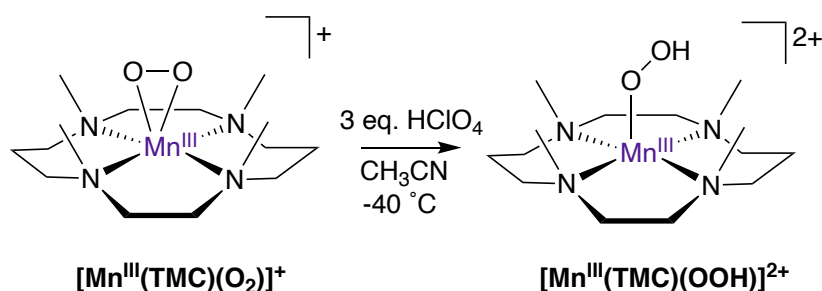


Figure 1.18: Conversion of a Mn^{III}-peroxide to a Mn^{III}-hydroperoxo upon addition of the acid HClO₄.⁹⁸

η^2 -peroxomanganese^{III} complexes are nucleophilic species capable of reacting with aldehydes, protons, acid anhydrides, and other metal centres. For example, reaction of the Mn^{III}-peroxide porphyrin complex with a benzoyl chloride or benzoyl anhydride resulted in the formation of an acylchloride Mn^{III}-peroxide that undergoes O-O bond scission to generate a Mn^V-oxo complex (Figure 1.19, A).^{103–105} In 2013, Groves and co-workers

synthesised a hydroxo-hydroperoxo-Mn^{III} complex supported by an electron deficient porphyrin system that was found to decay to form a Mn^V-oxo species.¹⁰⁶ This was of note as this system was found to mimic the “push-pull” mechanism of cytochrome P450 as the O-O heterolysis was coupled to the deprotonation of the *trans* OH⁻ ligand. The formation of high-valent Mn^V-oxo complexes from Mn^{III}-peroxides not supported by porphyrin ligands was less common.

The Mn^{III}-peroxide complex [Mn^{III}(O₂)(N4py)]⁺ was generated via reaction of the low-valent Mn^{II} complex [Mn^{II}(OTf)(N4py)]⁺ with O₂⁻ and then with the low-valent complex again to form a bis(μ -oxo)Mn^{III}Mn^{IV} complex [Mn^{III}Mn^{IV}(μ -O₂)(κ^4 -N4py)₂]⁺ (Figure 1.19, B).⁸⁸ This mimicked the postulated cycle in Ib RNRs whereby two Mn^{II} centres convert to a bis(μ -oxo)Mn^{III}Mn^{IV} complex utilising superoxide as an oxidant. Spectroscopic and computational studies validated the formation of the Mn^{III}-peroxide species supported by a tetradentate N4py ligand, however, it was not firmly established which of the pyridine groups (methylenepyridine vs pyridinylmethane) dissociated (Figure 1.19, B).⁸³ The mechanism of formation of the diamond core Mn^{III}Mn^{IV} species is still not understood. It was postulated that the nucleophilic Mn^{III}-peroxide species attacked the low-valent Mn^{II} species resulting in the formation of a dinuclear peroxo-bridged species that converted to the bis(μ -oxo)Mn^{III}Mn^{IV} complex. The bis(μ -oxo)Mn^{III}Mn^{IV} complex can also be formed by reacting [Mn^{II}(OTf)(N4py)]⁺ with H₂O₂ in the presence of a base or H₂O₂-urea, even at low-temperatures. This was unusual as treatment of the related [Mn^{II}(mL₅²)]⁺² and [Mn^{II}(imL₅²)]⁺² with H₂O₂ resulted in the formation of Mn^{III}-peroxide species.^{83,87,89} It is still unclear if a Mn^{III}-peroxide species was formed during the reaction of [Mn^{II}(OTf)(N4py)]⁺ with H₂O₂.

Due to the inherent nucleophilicity of Mn^{III}-peroxide complexes, they were capable of performing aldehyde deformylation. Mn^{III}-peroxide complexes supported by H₃bupa²⁻, H₂bpa²⁻, TMC, and L⁷py₂^R ligands (Figure 1.19, C).^{76,77,79,85,90,92} Nam and co-workers developed a series of Mn^{III}-peroxide complexes that contained an axial ligand *trans* to the peroxide group [Mn^{III}(O₂)(13-TMC)(X)] where X = CN⁻, NCS⁻, OOCCF₃⁻, and N₃⁻.⁷⁷ This allowed the effect of the electron-density on the peroxide group to be gauged by substitution of this axial ligand with a variety of electron donating and withdrawing groups. It was found that the nucleophilicity of the Mn^{III}-peroxide was markedly improved upon introduction of electron-donating axial ligands. Presumably the axial ligand improved the electron richness of the peroxide ligands and also may have changed the conformation from a side-on to end structure. This combination of effects worked to improve the reactivity of the complex.

Activation of Mn^{III}-peroxides towards electrophilic substrates has faced limited success. Despite this, the O-O bond of the [Mn^{III}(O₂)H₃bupa]⁻ complex was activated upon addition of a hydrogen-atom donor (Figure 1.19, D).¹⁰⁷ [Mn^{III}(O₂)H₃bupa]⁻ was found to be capable of reducing O₂ to H₂O in the presence of DPH or hydrazine acting as a reductant. [Mn^{II}H₂bupa]⁻ reacted with O₂ and 0.5 equivalents of DPH to form [Mn^{III}(O₂)H₃bupa]⁻. The Mn^{III}-peroxide accepted two H atoms from another equivalent of DPH and then undergoes O-O bond cleavage resulting in H₂O and a Mn^{III} intermediate described as a Mn^{III}-O and Mn^{III}-OOH hybrid that contained a strong hydrogen bond with the supporting ligand. The new Mn^{III} intermediate reacted with another 0.5 equivalent of DPH producing a second molecule of DPH and the regenerated [Mn^{II}H₂bupa]⁻ starting material. The activation of this Mn^{III}-peroxide complex using a strong reductant was very surprising as a related η^2 -peroxoiron^{III} adduct [Fe^{III}(O₂)(EDTA)]³⁻ was determined to be unreactive towards strong one-electron and two-electron reductants Na₂S₂O₄ and NaBH₄.⁹⁹ It was postulated the

difference in reactivity observed between peroxoiron and peroxomanganese complexes was due to the intrinsically lower potential of the $\text{Fe}^{\text{II/III}}$ couple in comparison to $\text{Mn}^{\text{II/III}}$ couple.

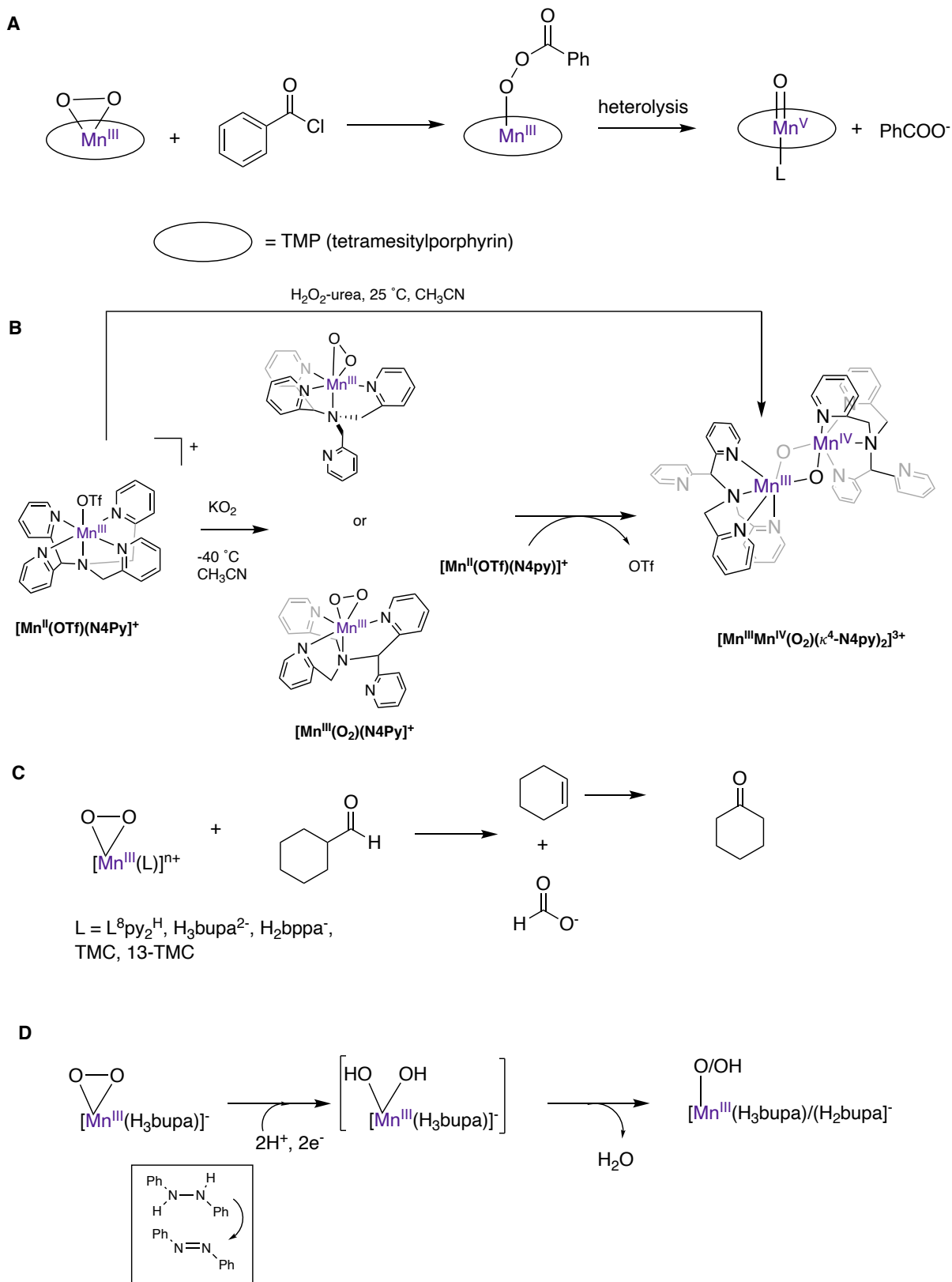


Figure 1.19: Chemical reactivity of Mn^{III} -peroxide adducts. OTf = trifluoromethanesulfonate.^{76,77,103–107,79,83,85,87–90,92}

1.4.3 Mixed Valent Mn^{II}Mn^{III}-Peroxides

Research in the area of mimicking the active site of class Ib RNRs, has been ongoing for decades. However, in 2018 a breakthrough was made when the first Mn^{II}Mn^{III}-peroxide complex was described by the McDonald group.¹⁰⁸ This new species, [Mn^{II}Mn^{III}(O₂)(N-Et-HPTB)]²⁺ (Figure 1.20), was formed via reaction of the low valent complex, [Mn^{II}Mn^{III}(N-Et-HPTB)(OCCH₃)₂](ClO₄) with superoxide at -40 °C. In 2019, the second Mn^{II}Mn^{III}-peroxide species was reported also by the McDonald group [Mn^{II}Mn^{III}(O₂)(BPMP)]²⁺, and was also generated via a similar procedure.¹⁰⁹ Both complexes displayed very similar electronic absorption features; an intense band between $\lambda_{max} = 440 - 460$ nm and a less intense feature $\lambda_{max} = 590 - 610$ nm (Table 1.8). These features are very similar to those displayed by Mn^{III}-peroxide complexes (Section 1.4.2, Table 1.7) and are typically associated with LMCT from the peroxide ligand to the Mn^{III} centre and d-d transitions.^{79,84,86}

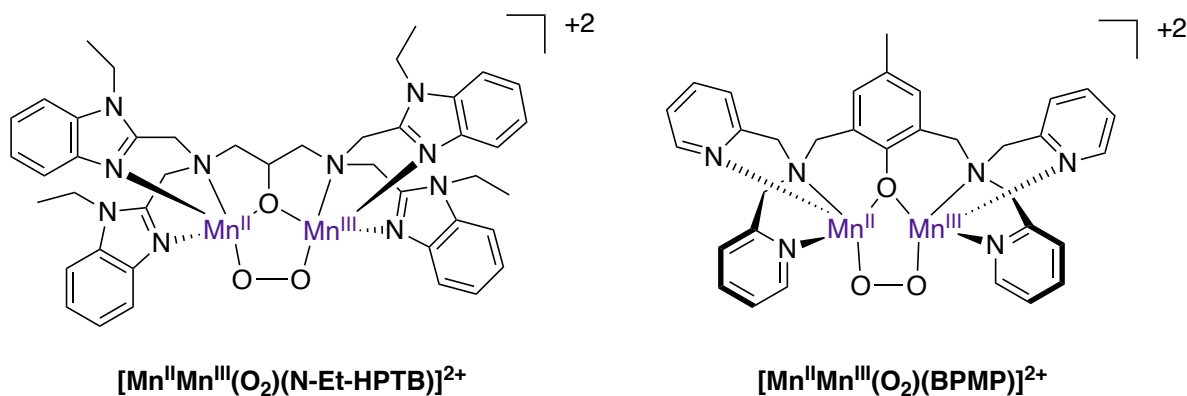


Figure 1.20: Structures of the Mn^{II}Mn^{III}-peroxide complexes synthesised by the McDonald group.^{108,109}

Complexes [Mn^{II}Mn^{III}(O₂)(N-Et-HPTB)]²⁺ and [Mn^{II}Mn^{III}(O₂)(BPMP)]²⁺ displayed EPR spectra with a 29-line signal centred around $g = 2$ and a range of 200 mT (Table 1.8). Both EPR signals were not resolved at EPR measurement temperatures > 15 K. The EPR features

of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ were reminiscent of previously characterised mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species, which typically display between 20-30 line signals centred around $g = 2$ and are only resolved below temperatures of 15 K (Section 1.4.1, Table 1.5).

Mn K-edge XANES studies of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ revealed an increase in edge energy of 1 eV relative to the Mn^{II}_2 precursor, indicative of a half-integer increase in average oxidation state of the two manganese centres.¹⁰⁸ Mn K-edge XANES was also performed on $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$, however, as this $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide was formed only in yields of 50 % an edge energy shift of just 0.5 eV was observed.¹⁰⁹ Extended X-ray absorption fine structure analysis (EXAFS) of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ were not performed due to the inability to obtain both species in a sufficiently high concentration. The difficulty in generating the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species in the high yields required for XAS analysis is also observed in Mn^{III} -peroxides.⁵⁴ $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ was found to be unreactive towards a variety of substrates, making it a poor electrophilic and nucleophilic oxidant. Interestingly, upon activation of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ with a proton-donor, a competent oxidant was formed capable of oxidising ferrocene to ferrocenium and activation of the O-H bonds of 1-hydroxy-2,2,6,6-tetramethyl-piperidine (TEMPO-H). Despite displaying similar spectroscopic properties, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ was found to be much more reactive than $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$, able to perform the deformylation of aldehydes. However, the new $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ was still determined to be a poor electrophilic oxidant. $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ displayed similar reactivity to those of Mn^{III} -peroxides which are capable of aldehyde deformylation and also found to be poor electrophiles.

The generation of mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species is of great interest to mimic the catalytic cycle of Ib RNRs. Both $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides were generated following reaction of

a Mn^{II}_2 complex with O_2^- indicating that these species could possibly be the identity of the unknown intermediate preceding $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ formation. Understanding the structures and reactivities of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides could provide great insight into the mechanism of Ib RNRs.

Table 1.8: Electronic absorption features and X-band EPR features of the two reported $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species.

Name	Electronic Absorption Features (λ_{max} / nm)	EPR Features (No. of Lines, g, width)	Reference
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{+2}$	460, 610	29, 1.96, 200 mT	108
$[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$	440, 590	22, 1.96, 200 mT	109

1.4.4 Bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ Complexes

$\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes are implicated as the intermediate capable of oxidation of the nearby tyrosine residue to a tyrosyl radical in Ib RNRs (Section 1.3.2, Figure 1.7). It was this tyrosyl radical species that initiated ribonucleotide reduction in the enzyme. Bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species were also implicated in the catalytic cycles of Mn-SOD, MnCAT, and the oxygen-evolving complex (OEC) in photosystem II.^{4,5,110} Understanding the spectroscopic properties and reactivity of small molecule bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes can shed light onto the catalytic cycles of these enzymes that cannot be studied *in vivo* due to the short lifetimes of the intermediates. Many bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes have been synthesised in the last 60 years and characterised via a variety of spectroscopic methods including XRD, UV-vis, EPR and infrared (IR) (Figure 1.21).

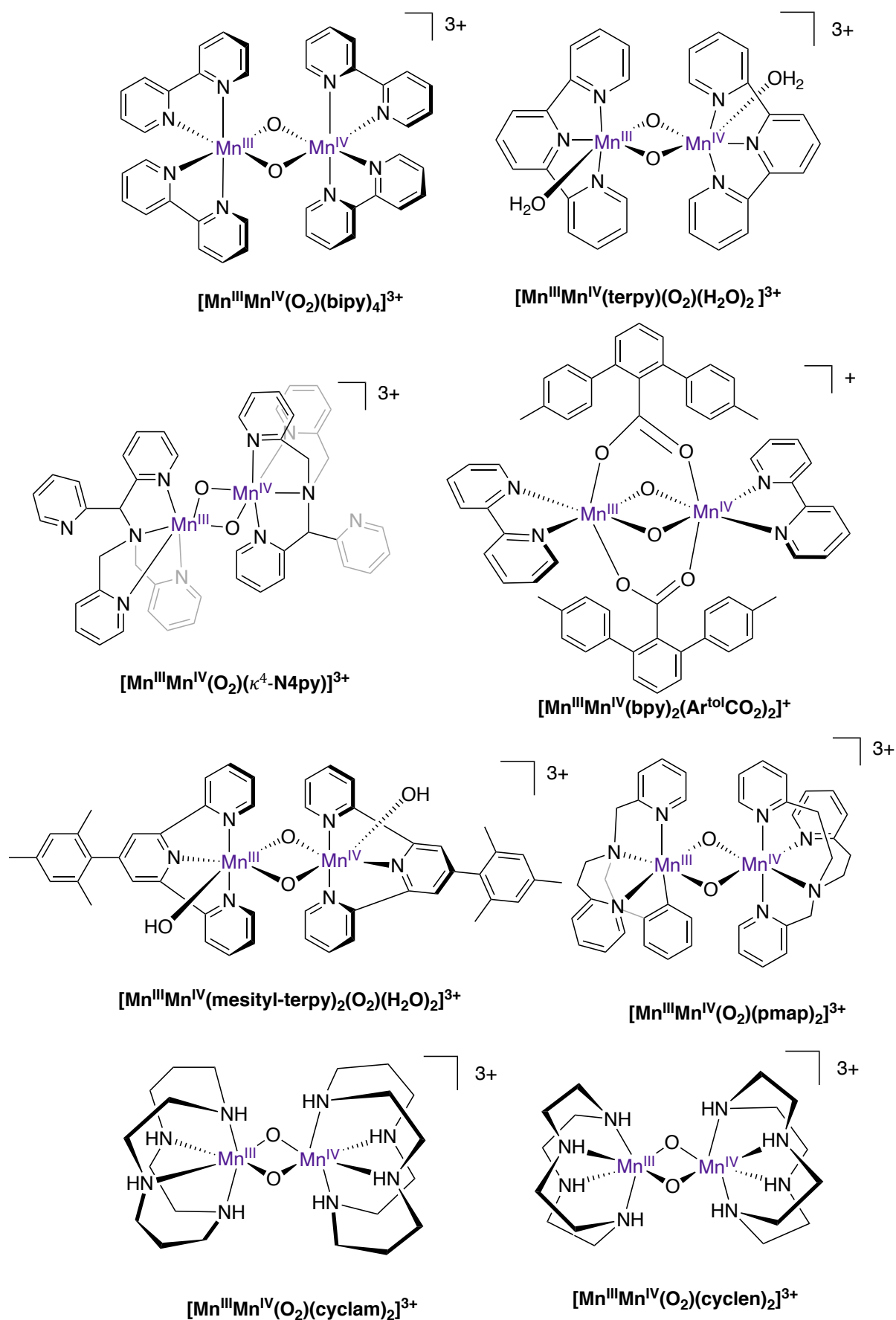


Figure 1.21: Selected structures of bis(μ-oxo)Mn^{III}Mn^{IV} complexes.^{88,111–116}

The first bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4](\text{S}_2\text{O}_8)_{1.5}$ was synthesised in 1960 by Nyholm and Turco (Figure 1.21).¹¹³ The $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex was isolated as greenish-black crystals following addition of molten bipy and $\text{K}_2\text{S}_2\text{O}_8$ to a suspension of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ in warm water. The corresponding perchlorate counterpart $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4](\text{ClO}_4) \cdot 2\text{H}_2\text{O}$ was obtained via a metathesis reaction. Two years later, Plaksin *et al.* reported the anhydrous analogue $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4](\text{ClO}_4)_3$.¹¹⁷ The crystal structure of the anhydrous complex suggested the formation of a mixed valent $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex based on the bond lengths and the significant Jahn-Teller distortion around the Mn^{III} centre. Interestingly, the Mn-Mn separation was found to be 2.716 Å for $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4](\text{ClO}_4)_3$, very similar to the Mn-Mn distance of 2.7 Å reported for the OEC.¹¹⁷ Since this discovery many more bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes have been analysed by XRD (Table 1.9). The Mn $\cdots$ Mn distances measured for the characterised bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ fall in range from 2.505 – 2.741 Å. The shortest distance Mn $\cdots$ Mn separation was found in $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{bpy})_2(\text{Ar}^{\text{tol}}\text{CO}_2)_2]^+$ reported by Armstrong *et al.*¹¹¹ The two bridging carboxylate moieties ligated to the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core of $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{bpy})_2(\text{Ar}^{\text{tol}}\text{CO}_2)_2]^+$ were postulated to be the reason for this short Mn $\cdots$ Mn distance. On the other hand, the longest Mn $\cdots$ Mn distance of 2.741 Å was found in $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{cyclam})_2]^{3+}$.¹¹⁸ The source of this increased length was proposed to be due to the large macrocyclic nature of the cyclam ligand which contained a 14-membered ring. The Mn $\cdots$ Mn distance was found to decrease when $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{cyclen})_2]^{3+}$ was synthesised using a related ligand; cyclen, the 12-membered ring version of cyclam (Table 1.9). This decrease in separation of the Mn ions was determined to be due to the decreased cavity size in the cyclen ligand, thus leading the metal atoms to sit more out of the plane in the 12-membered ring complex, compared to the 14-membered counterpart.

Table 1.9: Mn^{III} - Mn^{IV} separation for selected bis(μ -oxo) $Mn^{III}Mn^{IV}$ complexes.

$Mn^{III}Mn^{IV}$ Complex	$Mn^{III} \cdots Mn^{IV}$ [Å]	Reference
$[Mn^{III}Mn^{IV}(\mu-O)_2(bipy)_4]^{3+}$	2.716(1)	119
$[Mn^{III}Mn^{IV}(\mu-O)_2(phen)_4]^{3+}$	2.700(1)	119
$[Mn^{III}Mn^{IV}(\mu-O)_2(bispicen)_2]^{3+}$	2.659(2)	120
$[Mn^{III}Mn^{IV}(\mu-O)_2(bispicMe_2en)_2]^{3+}$	2.693(2)	121
$[Mn^{III}Mn^{IV}(\mu-O)_2(terpy)_2(CF_3CO_2)_2]^+$	2.727(5)	122
$[Mn^{III}Mn^{IV}(\mu-O)_2(tren)_2]^{3+}$	2.679(1)	123
$[Mn^{III}Mn^{IV}(\mu-O)_2(tmpa)_2]^{3+}$	2.643(1)	124
$[Mn^{III}Mn^{IV}(\mu-O)_2(cyclam)_2]^{3+}$	2.741(1)	118
$[Mn^{III}Mn^{IV}(\mu-O)_2(cyclen)_2]^{3+}$	2.694(1)	125
$[Mn^{III}Mn^{IV}(\mu-O)_2(terpy)_2(OH_2)_2]^{3+}$	2.273(3)	126
$[Mn^{III}Mn^{IV}(\mu-O)_2(pyclen)_2(OH_2)_2]^{3+}$	2.712(1)	127
$[Mn^{III}Mn^{IV}(bpy)_2(Ar^{tol}CO_2)_2]^+$	2.505(1)	111

Bis(μ -oxo) $Mn^{III}Mn^{IV}$ complexes all displayed only slight differences in their UV-vis features. Typically two features were observed - a characteristic absorption band between 520-570 nm assigned to $d-d$ transitions and bands in the near IR region between 590-700 nm may attributed to O to Mn charge transfer (Table 1.9).¹²⁸ The complex $[Mn^{III}Mn^{IV}(\mu-O)_2(terpy)_2(OH_2)_2]^{3+}$, for example, has electronic absorbance features at $\lambda_{max} = 553$ and 654 nm.¹²⁹ An exception to this trend was $[Mn^{III}Mn^{IV}(bpy)_2(Ar^{tol}CO_2)_2]^+$, which displayed remarkably different electronic absorption features to any other bis(μ -oxo) $Mn^{III}Mn^{IV}$ complex with a broad, low intensity shoulder at 760 nm.¹¹¹ The usual structure of $[Mn^{III}Mn^{IV}(bpy)_2(Ar^{tol}CO_2)_2]^+$, that contained a bis(μ -oxo) $Mn^{III}Mn^{IV}$ unit ligated by two bridged carboxylate ligands was postulated to the source of these usual features.

EPR spectroscopy was frequently used to assign a bis(μ -oxo) $Mn^{III}Mn^{IV}$ complexes, as these species displayed extremely characteristic spectra. Bis(μ -oxo) $Mn^{III}Mn^{IV}$ possess two metal centres; a Mn^{III} site with a spin of $S = 2$ and a Mn^{IV} site with a spin of $S = 3/2$. Both

centres are strongly antiferromagnetically coupled which results in an overall total spin $S_T = \frac{1}{2}$ ground state.¹¹² For this system the projection model predicted that the resulting hyperfine couplings (hfc's) to be: $A^{\text{III}} = 2a^{\text{III}}$ and $A^{\text{IV}} = -a^{\text{IV}}$ where a^{III} and a^{IV} are the intrinsic hfc's of Mn^{III} and Mn^{IV} respectively. An assumption of $a^{\text{III}} = a^{\text{IV}}$ and isotropic g tensor was made due to the small ^{55}Mn -hfc anisotropy in $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ systems to simplify the calculations.¹¹⁵ This resulted in the characteristic $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ signal in the EPR spectrum whereby much of the possible 36 lines overlay, generating the 16-line signal. The 16-line spectra was considered a “fingerprint” for $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. A 16-line spectrum indicated that valences of individual Mn atoms are trapped, i.e. a “true” $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$. Theoretically, if there was delocalisation of the unpaired electron over the highest occupied d-orbital of each Mn atom or a fast electron hopping on the timescale of the EPR experiment a broken $\text{Mn}^{3.5}\text{Mn}^{3.5}$ would be obtained that displayed an 11-line signal with a smaller width compared to the signal of the trapped $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ case, however, experimentally this has never been observed.⁶⁸

Many synthetic $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes have been analysed by EPR spectroscopy. The majority of $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes displayed a 16-line signal centred around $g = 2$ (Table 1.9). For example, $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\kappa^4\text{-N4py})_2]^{3+}$, $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{bpy})_2(\text{Ar}^{\text{tol}}\text{CO}_2)_2]^+$, and $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{cyclam})_2]^{3+}$ all displayed a 16-line signal centred around $g = 2$ even though each complex was supported by different ligand systems (Figure 1.20).^{88,111,118} The $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core in $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\kappa^4\text{-N4py})_2]^{3+}$ was ligated by small molecule tetradentate N4py, while in $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{bpy})_2(\text{Ar}^{\text{tol}}\text{CO}_2)_2]^+$ the core was bridged by two carboxylate ligands as well as the bidentate bipyridine (bpy) ligands, and the $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{cyclam})_2]^{3+}$ was ligated by a 14-membered macrocyclic ligand. To emphasise the difference in the ligand environments, these systems are so different that $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{cyclam})_2]^{3+}$ and

$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{bpy})_2(\text{Ar}^{\text{tol}}\text{CO}_2)_2]^+$ produced the longest and shortest Mn ... Mn distances respectively.^{111,118} Despite this, all three complexes displayed the same 16-line signal. Therefore, it appeared the EPR signals of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes are not very sensitive to changes in the ligand environment.

Bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes also displayed very narrow EPR spectral widths, typically between 130 – 160 mT (Table 1.9). Dismukes and co-workers postulated the short range as a means to distinguish $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ systems from $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ systems which displayed much larger spectral widths, with comparable ligands.⁵⁸ The reason for this disparity in width was because the spin-coupling coefficients that multiple the hfc constants are larger for $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes than $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$.⁶⁸ Another distinction between the EPR's of $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ and $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes was the temperature dependencies. The EPR spectra of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ were resolved at measurement temperatures from 2 K to 140 K, for example, the complex $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{PCINOL})_2]^+$, displays a sharp, well-defined 16-line signal centred at $g = 2$ at 140 K.¹³⁰ In contrast, the EPR signals of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes are only completely resolved at measurement temperatures <15 K.¹⁰⁹

Table 1.10: Electronic absorption and X-band features for reported $Mn^{III}Mn^{IV}$ complexes.

$Mn^{III}Mn^{IV}$	Electronic Absorption Features $\lambda_{max}/ nm (\epsilon / M^{-1} cm^{-1})$	EPR Features No. of Lines, g, width	Ref
$[Mn^{III}Mn^{IV}(\mu-O)_2(\kappa^4-N4py)_2]^{3+}$	565 (830), 667 (680)	16, 2, 140 mT	88
$[Mn^{III}Mn^{IV}(\mu-O)_2(pmap)_2]^{3+}$	556 (ND), 662 (ND)	16, 2, 130 mT	114
$[Mn^{III}Mn^{IV}(\mu-O)_2(bisimMe_2en)_2]^{3+}$	419 (950), 536 (428), 644 (304)	16, 2, 135 mT	131
$[Mn^{III}Mn^{IV}(\mu-O)_2(bispicMe_2en)_2]^{3+}$	659 (458), 552 (480), 430 (1155), 380 (1472)	-	121
$[Mn^{III}Mn^{IV}(\mu-O)_2(tren)_2]^{3+}$	-	16, 2, 130 mT	123
$[Mn^{III}Mn^{IV}(\mu-O)_2(tpa)_2]^{3+}$	660 (500), 559 (621), 505 (650), 439 (1250), 382 (1400)	16, 2, 130 mT	112
$[Mn^{III}Mn^{IV}(\mu-O)_2(bpg)_2]^{3+}$	640 (420), 545 (550), 434 (1250), 380 (1480)	16, 2, 130 mT	112
$[Mn^{III}Mn^{IV}(\mu-O)_2(pda)_2]^{3+}$	590 (450), 537 (600), 413 (1550), 375 (ND)	16, 2, 130 mT	112
$[Mn^{III}Mn^{IV}(\mu-O)_2(cyclam)_2]^{3+}$	556 (750), 646 (760)	16, 2, -	118
$[Mn^{III}Mn^{IV}(\mu-O)_2(cyclen)_2]^{3+}$	553 (ND), 644 (ND)	-	132
$[Mn^{III}Mn^{IV}(\mu-O)_2(bpy)_4]^{3+}$	525 (530), 555(455), 684 (561)	16, 2, -	133,134
$[Mn^{III}Mn^{IV}(\mu-O)_2(phen)_4]^{3+}$	525 (509), 555 (511), 684 (553)	16, 2, -	133,134
$[Mn^{III}Mn^{IV}(\mu-O)_2(phen)_4](PF_6)_3 \cdot CH_3CN$	523 (580), 550 (460), 680 (550), 800 (ND)	-	119
$[Mn^{III}Mn^{IV}(bpy)_2(Ar^{tol}CO_2)_2]^+$	Shoulder at ~ 760 nm	16, 2, 135 mT	111
$[Mn^{III}Mn^{IV}(\mu-O)_2(tmpa)_2]^{3+}$	443 (1490), 561 (760), 658 (620)	16, 2, 150 mT	135
$[Mn^{III}Mn^{IV}(\mu-O)_2(N,N-bispicen)_2]^{3+}$	433 (1080), 555 (511), 645 (450)	16, 2, 130 mT	136
$[Mn^{III}Mn^{IV}(\mu-O)_2(\mu-O_2CMe)L_{M_2}]^{2+}$	259 (21000), 400 (1100), 440 (750), 548 (300), 640 (350), 800 (150)	16, 2, 135 mT	137
$[Mn^{III}Mn^{IV}(\mu-O)_2(2-OH)(3,5-Cl_2Sal)pn)_2]^+$	400 (10000)	16, 2, 160 mT	55
$[Mn^{III}Mn^{IV}(\mu-O)_2(terpy)_2(OH_2)_2]^{3+}$	553 (678), 654 (585)	-	129
$[Mn^{III}Mn^{IV}(\mu-O)_2(terpy)_2(CF_3CO_2)_2]^+$	278 (31000), 324 (25000), 550 (725), 620 (650)	-	122
$[Mn^{III}Mn^{IV}(\mu-O)_2(py-terpy)(OH_2)]^{3+}$	552 (612), 655 (608)	-	128

$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{R-terpy})_2(\text{OH})_2]^{3+}$			
R = Cl	552 (610), 658 (603)	-	116
R = H	551 (605), 655 (600)	-	
R = MeS	552 (616), 655 (604)	-	
R = Me	551 (619), 655 (608)	-	
R = EtO	552 (620), 655 (598)	-	
R = PrO	552 (610), 655 (603)	-	
R = MeO	552 (619), 655 (603)	-	
R = BuO	551 (623), 650 (610)	-	

IR spectroscopy was another useful technique for the assignment of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes as the Mn-O-Mn stretch of the diamond core was IR active. The asymmetric stretch (ν_{as}) of the Mn-O-Mn unit falls around 700 cm^{-1} (Table 1.11). Yagi and co-workers performed studies to understand how the ligand environment effected the bis(μ -oxo) core.¹¹⁶ Substitution of the R group of the complex $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{R-terpy})_2(\text{OH})_2]^{3+}$ with a variety of electron donating and withdrawing groups resulted in a shift of the ν_{as} stretch. The Mn-O-Mn unit was significantly strengthened when electron-withdrawing Cl groups were utilised as evidenced by the unusually high ν_{as} stretch of 712 cm^{-1} . On the other hand, the electron donating groups such as MeO and EtO did not have as much of strengthening effect on the Mn-O-Mn unit. Yagi postulated that the ionic bonding properties between the anionic $\mu\text{-O}$ and cationic Mn centres may be enhanced by the electron-withdrawing Cl but not so in the case of the electron donating groups. The symmetric stretch (ν_{s}) of the Mn-O-Mn unit can also be seen via IR spectroscopy, however, it was very difficult to detect and has only been observed in a handful of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes.^{112,132} This is because the ν_{s} of the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core does not result in a change in dipole moment and therefore does not appear in an IR spectrum. In the complexes where ν_{s} is visible the core contains an element of asymmetry, breaking the centrosymmetry of the core inducing a dipole moment during the symmetric

stretch and even then, the signal produced is weak. The ν_s and ν_{as} stretch are sensitive to $^{16}\text{O}/^{18}\text{O}$ isotope substitution. For example, for the complex $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O}_2)(\text{tpa})_2]^{3+}$ the ν_s and ν_{as} at 703 and 611 cm^{-1} respectively were both found to shift by -25 cm^{-1} upon substitution with ^{18}O .¹¹²

Table 1.11: IR data for bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes.

$\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ Complex	IR Data Mn-O-Mn Stretch (ν_{as} cm^{-1})	IR Data Mn-O-Mn Stretch (ν_s cm^{-1})	Reference
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{tren})_2]^{3+}$	694	-	123
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{tpa})_2]^{3+}$	703	611	112
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bpg})_2]^{3+}$	697	605	112
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{cyclen})_2]^{3+}$	690	570	132
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{phen})_4](\text{PF}_6)_3 \cdot \text{CH}_3\text{CN}$	686	-	119
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{terpy})_2(\text{OH}_2)_2]^{3+}$	706	-	129
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{py-terpy})(\text{OH}_2)]^{3+}$	700	-	128
$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{R-terpy})_2(\text{OH})_2]^{3+}$			
R = Cl	712	-	116
R = H	698	-	
R = MeS	700	-	
R = Me	702	-	
R = EtO	705	-	
R = PrO	703	-	
R = MeO	703	-	

Bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species were implicated in the catalytic cycles of Mn CAT, Ib RNRs, and the OEC, therefore, reactivity studies of synthetic $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes were focused on attempting to replicate the activity observed in these enzymes. For example, complexes

$[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4]^{3+}$ and $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{phen})_4]^{3+}$ were capable of catalysing water oxidation when suspended heterogeneously in water in the presence of the chemical oxidant Ce^{IV} , similar to the reactivity observed in the OEC.¹³⁸ $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4]^{3+}$ was noted to be a more efficient catalyst compared to $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{phen})_4]^{3+}$ which was consistent with the cyclic voltammetry studies that revealed $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{bipy})_4]^{3+}$ was the easier to oxidise of the two.¹³⁸ The complex $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{tmpa})_2](\text{ClO}_4)_3$ was tested for its ability to mimic the superoxidised Mn-CAT (III,IV) and found successfully able to convert H_2O_2 to O_2 .¹¹⁰ A substrate versus initial velocity plot of the reaction between $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{tmpa})_2](\text{ClO}_4)_3$ and H_2O_2 revealed a saturation behaviour that was modelled using the Hill equation to obtain kinetic parameters. Han and co-workers postulated that substrate-activation of the catalyst was the origin of this saturation kinetics and that $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{O}_2)(\text{tmpa})_2](\text{ClO}_4)_3$ acted as a pre-catalyst. Finally, Jackson *et al.* demonstrated that both bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{N4py})]^{3+}$ and $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\mu\text{-O})_2(\text{DMMN4py})]^{3+}$ were capable of reacting with the O-H bond of 2,4-di-*tert*-butylphenol, mimicking the reactivity observed in the catalytic cycle of Ib RNRs whereby an O-H bond was oxidised to the oxygen based radical species.¹³⁹ Therefore, it can be seen that $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes were very versatile species capable of a wide variety of chemistry.

1.5.1 Conclusions

RNRs are enzymes responsible for the conversion of ribonucleotides to the corresponding deoxyribonucleotides, essential precursors for DNA synthesis and repair in all organisms. In the last twenty years considerable leaps in understanding RNRs have been achieved. Despite this many unanswered questions linger around the enzyme and even still many exciting discoveries have yet to be made. Even in the last five years, two new subclasses

of enzyme have been discovered with possibly more yet to be uncovered. Many advances especially have been made in understanding the mechanisms of these subclasses, however, holes in our mechanistic knowledge exist due to transient intermediates that cannot be trapped due to their fleeting lifetimes. Mimicking the catalytic cycle using synthetic mimics of the enzyme's active site may help shed some light on the structures and mechanisms of these unknown intermediates.

For example, it was only shown in 2013 that the activating oxidant for class Ib RNR was superoxide (O_2^-), not dioxygen (O_2), as was assumed due to the Ib's similarity to the Ia subclass. Stubbe and co-workers demonstrated that following reaction of O_2^- with the Mn^{II}_2 active site and a proton source an unknown intermediate species was generated that rapidly interconverted to generate a high valent $Mn^{III}Mn^{IV}$ intermediate. This $Mn^{III}Mn^{IV}$ species was responsible for the oxidation of a nearby tyrosine to a tyrosyl radical, initiating ribonucleotide reduction. Despite this, little experimental evidence has been provided for this catalytic cycle. Only one EPR spectrum supported the formation of a $Mn^{III}Mn^{IV}$ intermediate as the active oxidant and no experimental evidence exists for the unknown intermediate following reaction of Mn^{II}_2 and O_2^- that has never been trapped.

Mn^{III} -peroxides have been implicated in the catalytic cycle of a variety of enzymes such as Mn-Cat and Mn-SOD. Interestingly, Mn^{III} -peroxides have been found to convert to bis(μ -oxo) $Mn^{III}Mn^{IV}$ complexes, making them likely candidates for unknown intermediate in the catalytic cycle of Ib RNRs. The first $Mn^{II}Mn^{III}$ -peroxide was synthesised recently following reaction of a Mn^{II}_2 species with O_2^- , mimicking the first step in the cycle of Ib RNRs. Therefore, $Mn^{II}Mn^{III}$ -peroxides could be a promising candidate for the unknown intermediate species. As seen in figure 1.22, activation of a $Mn^{II}Mn^{III}$ -peroxide species may allow us to access a bis(μ -oxo) $Mn^{III}Mn^{IV}$ from which we can probe the radical oxidation mechanism of Ib RNRs. While many studies have been performed on Mn^{III} -peroxides and

bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes, the complete reactivity of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides and what they can tell us about the catalytic cycle of Ib RNRs is still yet to be explored. This was to be the focus of this project.

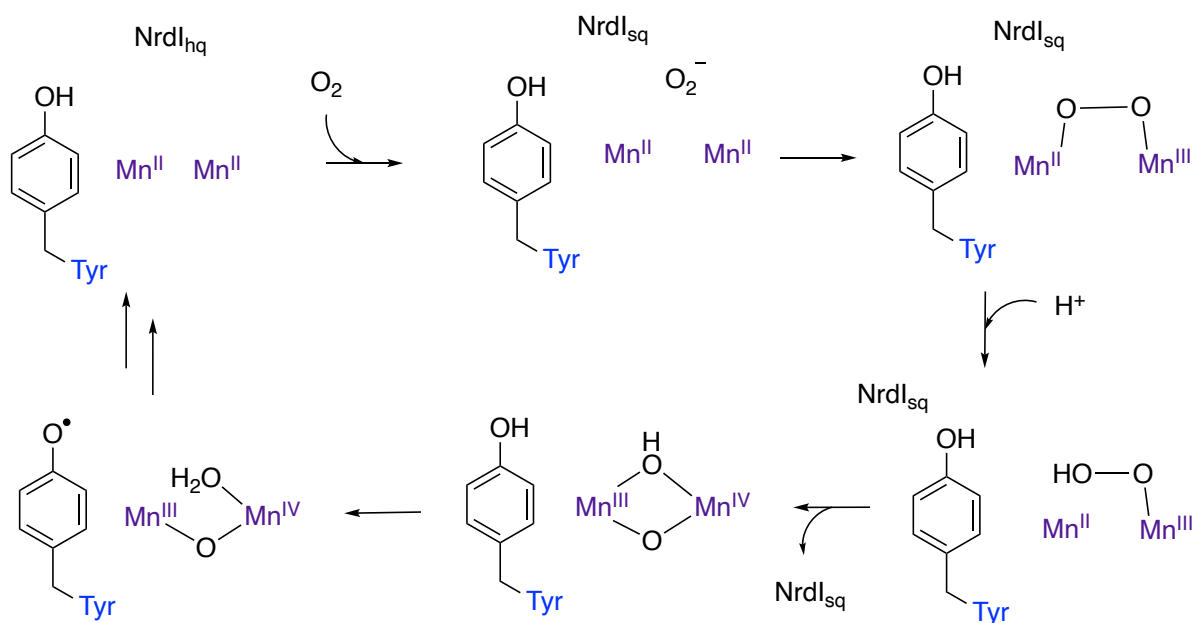


Figure 1.22: Proposed catalytic cycle for class Ib RNRs (NrdI_{hq} = Flavodoxin hydroquinone, NrdI_{sq} = Flavodoxin semiquinone).

1.5.2 Aims

The aim of this project is to completely mimic the catalytic cycle of class Ib RNRs using biomimetic model Mn^{II}_2 complexes. It has been demonstrated by Stubbe and co-workers that superoxide (O_2^-) was employed as the activating oxidant which reacts with the Mn^{II}_2 active site to form a possible $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide/hydroperoxo intermediate species.³⁵ This mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ intermediate rapidly converted to a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex with a possible proton on the diamond core. This $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species was implicated as the active oxidant responsible for the one-electron oxidation of a nearby tyrosine to a tyrosyl radical. EPR evidence is available to support the formation of the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species as the active species, however, the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide/hydroperoxide intermediate has never

been trapped and no spectroscopic information is available of this species. Hence, it is of great interest to understand the reactivity of Mn^{II}_2 with O_2^- in order to gain insight to this transient intermediate. McDonald and co-workers have made strides in this area by generating two $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species upon reaction of Mn^{II}_2 complexes with chemically generated O_2^- .^{108,109} However, neither of the new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides were capable of mimicking the O-H bond reactivity observed in Ib RNRs. Therefore, the main aim of this project is to attempt to activate the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides in order to achieve the reactivity observed in the catalytic cycle of Ib RNRs and through this gain insight into the mechanism of the enzyme (Figure 1.23). The reactivity of any new species formed with O-H bond containing substrates will be tested in order to assess its ability to mimic the activity of Ib RNRs. From this, we aim to understand more about the oxidation step in the enzyme and how it proceeds *in vivo*.



Figure 1.23: Activation of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide using proton sources, heat, and Lewis acids.

The first step of this will be the generation and optimisation of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide previously synthesised by the McDonald group. We will attempt to activate the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide using protons sources, heat, and Lewis acids. We will also attempt to synthesise new dinuclear Mn^{II}_2 complexes in order to generate new, more reactive $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species. Any new manganese species formed will be characterised via a variety of techniques such as mass spectrometry, resonance Raman, electronic absorption, XANES and EPR spectroscopies. Once characterised, the reactivity of any new intermediates will be investigated to see if they are capable of performing O-H bond oxidation, similar to that observed in the catalytic cycle of Ib RNRs.

References

- (1) Kakegawa, T.; Noda, M.; Nannri, H. *Resour. Geol.* **2002**, 52 (2), 83–89.
- (2) Yannone, S. M.; Hartung, S.; Menon, A. L.; Adams, M. W. W.; Tainer, J. A. *Current Opinion in Biotechnology*. February 2012, pp 89–95.
- (3) Robinson, N. J.; Glasfeld, A. *Journal of Biological Inorganic Chemistry*. Springer April 24, 2020, pp 1–3.
- (4) Younus, H. *Int. J. Health Sci. (Qassim)*. **2018**, 12 (3), 88–93.
- (5) Whittaker, J. W. *Archives of Biochemistry and Biophysics*. Academic Press Inc. September 15, 2012, pp 111–120.
- (6) Cotruvo, J. A.; Stubbe, J. *Annu. Rev. Biochem.* **2011**, 80 (1), 733–767.
- (7) Eriksson, M.; Jordan, A.; Eklund, H. *Biochemistry* **1998**, 37 (38), 13359–13369.
- (8) Salgado, V.; Rio Piedras, R.; Gaur, K.; Pérez Otero, S. C.; Benjamín-Rivera, J. A.; Rodríguez, I.; Loza-Rosas, S. A.; Vázquez Salgado, A. M.; Akam, E. A.; Hernández-Matias, L.; Sharma, R. K.; Alicea, N.; Kowaleff, M.; Washington, A. V.; Astashkin, A. V.; Tomat, E.; Tinoco, A. D.; Rio Piedras, R.; Juan, S.; Rico, P. *JACS Au* **2021**.
- (9) Torrents, E. *Front. Cell. Infect. Microbiol.* **2014**, 4 (52), 1-9.
- (10) Sintchak, M. D.; Arjara, G.; Kellogg, B. A.; Stubbe, J. A.; Drennan, C. L. *Nat. Struct. Biol.* **2002**, 9 (4), 293–300.
- (11) Nordlund, P.; Reichard, P. *Annu. Rev. Biochem.* **2006**, 75 (1), 681–706.
- (12) Stubbe, J. A.; Ge, J.; Yee, C. S. *Trends Biochem. Sci.* **2001**, 26 (2), 93–99.
- (13) Minnihan, E. C.; Nocera, D. G.; Stubbe, J. A. *Acc. Chem. Res.* **2013**, 46 (11), 2524–2535.
- (14) Ge, J.; Yu, G.; Ator, M. A.; Stubbe, J. A. *Biochemistry* **2003**, 42 (34), 10071–10083.
- (15) Rozman Grinberg, I.; Lundin, D.; Hasan, M.; Crona, M.; Jonna, V. R.; Loderer, C.; Sahlin, M.; Markova, N.; Borovok, I.; Berggren, G.; Hofer, A.; Logan, D. T.; Sjöberg, B. M. *Elife* **2018**, 7.
- (16) Zhong, J.; Reinhardt, C. R.; Hammes-Schiffer, S. *J. Am. Chem. Soc.* **2022**, 144 (16), 7208–7214.
- (17) Seyedsayamdost, M. R.; Yee, C. S.; Reece, S. Y.; Nocera, D. G.; Stubbe, J. A. *J. Am. Chem. Soc.* **2006**, 128 (5), 1562–1568.
- (18) Seyedsayamdost, M. R.; Xie, J.; Chan, C. T. Y.; Schultz, P. G.; Stubbe, J. *J. Am. Chem. Soc.* **2007**, 129 (48), 15060–15071.
- (19) Greene, B. L.; Greene, B. L.; Greene, B. L.; Kang, G.; Cui, C.; Cui, C.; Bennati, M.; Bennati, M.; Nocera, D. G.; Drennan, C. L.; Drennan, C. L.; Drennan, C. L.; Stubbe, J. A.; Stubbe, J. A. *Annu. Rev. Biochem.* **2020**, 89, 45–75.
- (20) Nordlund, P.; Sjöberg, B. M.; Eklund, H. *Nature* **1990**, 345 (6276), 593–598.
- (21) Nordlund, P.; Eklund, H. *J. Mol. Biol.* **1993**, 232 (1), 123–164.
- (22) Atkin, C. L.; Thelander, L.; Reichard, P.; Lang, G. *J. Biol. Chem.* **1973**, 248 (21), 7464–7472.
- (23) Meichsner, S. L.; Kutin, Y.; Kasanmascheff, M. *Angew. Chemie - Int. Ed.* **2021**, 60 (35), 19155–19161.
- (24) Ravi, N.; Huynh, B. H.; Edmondson, D. E.; Bollinger, J. M.; Stubbe, J. A. *J. Am. Chem. Soc.* **1994**, 116 (18), 8007–8014.
- (25) Dassama, L. M. K.; Silakov, A.; Krest, C. M.; Calixto, J. C.; Krebs, C.; Bollinger, J. M.; Green, M. T. *J. Am. Chem. Soc.* **2013**, 135 (45), 16758–16761.
- (26) Wu, C. H.; Jiang, W.; Krebs, C.; Stubbe, J. *Biochemistry* **2007**, 46 (41), 11577–11588.

- (27) Cotruvo, J. A.; Stubbe, J. *Biochemistry* **2010**, *49* (6), 1297–1309.
- (28) Cotruvo, J. A.; Stubbe, J. *Metallomics* **2012**, *4* (10), 1020–1036.
- (29) Boal, A. K.; Cotruvo, J. A.; Stubbe, J.; Rosenzweig, A. C. *Science* **2010**, *329* (5998), 1526–1530.
- (30) Boal, A. K.; Cotruvo, J. A.; Stubbe, J.; Rosenzweig, A. C. *Biochemistry* **2012**, *51* (18), 3861–3871.
- (31) Cox, N.; Ogata, H.; Stolle, P.; Reijerse, E.; Auling, G.; Lubitz, W. *J. Am. Chem. Soc.* **2010**, *132* (32), 11197–11213.
- (32) Cox, N.; Ogata, H.; Stolle, P.; Reijerse, E.; Auling, G.; Lubitz, W. *J. Am. Chem. Soc.* **2010**, *132* (32), 11197–11213.
- (33) Rose, H. R.; Ghosh, M. K.; Maggiolo, A. O.; Pollock, C. J.; Blaesi, E. J.; Hajj, V.; Wei, Y.; Rajakovich, L. J.; Chang, W. C.; Han, Y.; Hajj, M.; Krebs, C.; Silakov, A.; Pandelia, M. E.; Bollinger, J. M.; Boal, A. K. *Biochemistry* **2018**, *57* (18), 2679–2693.
- (34) Roca, I.; Torrents, E.; Sahlin, M.; Gibert, I.; Sjöberg, B. M. *J. Bacteriol.* **2008**, *190* (14), 4849–4858.
- (35) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, *135* (10), 4027–4039.
- (36) Diril, H.; Chang, H. R.; Zhang, X.; Larsen, S. K.; Potenza, J. A.; Pierpont, C. G.; Schugar, H. J.; Isied, S. S.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1987**, *109* (20), 6207–6208.
- (37) Diril, H.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S.; Chang, H. R.; Nilges, M. J.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, *111* (14), 5102–5114.
- (38) Sano, Y.; Weitz, A. C.; Ziller, J. W.; Hendrich, M. P.; Borovik, A. S. *Inorg. Chem.* **2013**, *52* (18), 10229–10231.
- (39) Zheng, M.; Khangulov, S. V.; Dismukes, G. C.; Barynin, V. V. *Inorg. Chem.* **1994**, *33* (2), 382–387.
- (40) Jiang, W.; Yun, D.; Saleh, L.; Barr, E. W.; Xing, G.; Hoffart, L. M.; Maslak, M. A.; Krebs, C.; Bollinger, J. M. *Science* **2007**, *316* (5828), 1188–1191.
- (41) Dassama, L. M. K.; Krebs, C.; Bollinger, J. M.; Rosenzweig, A. C.; Boal, A. K. *Biochemistry* **2013**, *52* (37), 6424–6436.
- (42) Voegtli, W. C.; Sommerhalter, M.; Saleh, L.; Baldwin, J.; Bollinger, J. M.; Rosenzweig, A. C. *J. Am. Chem. Soc.* **2003**, *125* (51), 15822–15830.
- (43) Yang, Y. S.; Baldwin, J.; Ley, B. A.; Bollinger, J. M.; Solomon, E. I. *J. Am. Chem. Soc.* **2000**, *122* (35), 8495–8510.
- (44) Martinie, R. J.; Blaesi, E. J.; Krebs, C.; Martin Bollinger, J.; Silakov, A.; Pollock, C. J. *J. Am. Chem. Soc.* **2017**, *139* (5), 1950–1957.
- (45) Jiang, W.; Hoffart, L. M.; Krebs, C.; Bollinger, J. M. *Biochemistry* **2007**, *46* (30), 8709–8716.
- (46) Covès, J.; Delon, B.; Climent, I.; Sjöberg, B. -M; Fontecave, M. *Eur. J. Biochem.* **1995**, *233* (1), 357–363.
- (47) Jiang, W.; Saleh, L.; Barr, E. W.; Xie, J.; Gardner, M. M.; Krebs, C.; Bollinger, J. M. *Biochemistry* **2008**, *47* (33), 8477–8484.
- (48) Jiang, W.; Xie, J.; Nørgaard, H.; Bollinger, J. M.; Krebs, C. *Biochemistry* **2008**, *47* (15), 4477–4483.
- (49) Martinie, R. J.; Blaesi, E. J.; Bollinger, J. M.; Krebs, C.; Finkelstein, K. D.; Pollock, C. J. *Angew. Chemie - Int. Ed.* **2018**, *57* (39), 12754–12758.
- (50) Rose, H. R.; Maggiolo, A. O.; McBride, M. J.; Palowitch, G. M.; Pandelia, M. E.; Davis, K. M.; Yennawar, N. H.; Boal, A. K. *Biochemistry* **2019**, *58* (14), 1845–1860.

- (51) Blaesi, E. J.; Palowitch, G. M.; Hu, K.; Kim, A. J.; Rose, H. R.; Alapati, R.; Lougee, M. G.; Kim, H. J.; Taguchi, A. T.; Tan, K. O.; Laremore, T. N.; Griffin, R. G.; Krebs, C.; Matthews, M. L.; Silakov, A.; Bollinger, J. M.; Allen, B. D.; Boal, A. K. *Proc. Natl. Acad. Sci.* **2018**, *115* (40), 10022–10027.
- (52) Srinivas, V.; Lebrette, H.; Lundin, D.; Kutin, Y.; Sahlin, M.; Lerche, M.; Eirich, J.; Branca, R. M. M.; Cox, N.; Sjöberg, B. M.; Högbom, M. *Nature* **2018**, *563* (7731), 416–420.
- (53) Ruskoski, T. B.; Boal, A. K. *J. Biol. Chem.* **2021**, *297* (4), 101137.
- (54) Leto, D. F.; Jackson, T. A. *J. Biol. Inorg. Chem.* **2014**, *19* (1), 1–15.
- (55) Gelasco, A.; Kirk, M. L.; Kampf, J. W.; Pecoraro, V. L. *Inorg. Chem.* **1997**, *36* (9), 1829–1837.
- (56) Khangulov, S. V.; Pessiki, P. J.; Barynin, V. V.; Ash, D. E.; Dismukes, G. C. *Biochemistry* **1995**, *34* (6), 2015–2025.
- (57) Chang, H. R.; Nilges, M. J.; Hendrickson, D. N.; Diril, H.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S. *J. Am. Chem. Soc.* **1988**, *110* (2), 625–627.
- (58) Dismukes, G. C.; Sheats, J. E.; Smeeal, J. A. *J. Am. Chem. Soc.* **1987**, *109* (23), 7202–7203.
- (59) Dubois, L.; Xiang, D. F.; Tan, X. S.; Pécaut, J.; Jones, P.; Baudron, S.; Le Pape, L.; Latour, J. M.; Baffert, C.; Chardon-Noblat, S.; Collomb, M. N.; Deronzier, A. *Inorg. Chem.* **2003**, *42* (3), 750–760.
- (60) Buchanan, R. M.; Oberhausen, K. J.; Richardson, J. F. *Inorg. Chem.* **1988**, *27* (6), 971–973.
- (61) Pessiki, P. J.; Dismukes, G. C. *J. Am. Chem. Soc.* **1994**, *116* (3), 898–903.
- (62) Teutloff, C.; Schäfer, K. O.; Sinnecker, S.; Barynin, V.; Bittl, R.; Wieghardt, K.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **2005**, *43* (SPEC. ISS.), S51–S64.
- (63) Cox, N.; Ames, W.; Epel, B.; Kulik, L. V.; Rapatskiy, L.; Neese, F.; Messinger, J.; Wieghardt, K.; Lubitz, W. *Inorg. Chem.* **2011**, *50* (17), 8238–8251.
- (64) Wieghardt, K.; Bossek, U.; Ventur, D.; Weiss, J. *J. Chem. Soc. - Ser. Chem. Commun.* **1985**, *0* (6), 347–349.
- (65) Singh, R.; Haukka, M.; McKenzie, C. J.; Nordlander, E. *Eur. J. Inorg. Chem.* **2015**, *2015* (21), 3485–3492.
- (66) Cox, N.; Ames, W.; Epel, B.; Kulik, L. V.; Rapatskiy, L.; Neese, F.; Messinger, J.; Wieghardt, K.; Lubitz, W. *Inorg. Chem.* **2011**, *50* (17), 8238–8251.
- (67) Boelrijk, A. E. M.; Khangulov, S. V.; Dismukes, G. C. *Inorg. Chem.* **2000**, *39* (14), 3009–3019.
- (68) Teutloff, C.; Schäfer, K. O.; Sinnecker, S.; Barynin, V.; Bittl, R.; Wieghardt, K.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **2005**, *43* (SPEC. ISS.), 51–64.
- (69) Darovsky, A.; Kezerashvili, V.; Coppens, P.; Weyhermüller, T.; Hummel, H.; Wieghardt, K. *Inorg. Chem.* **1996**, *35* (24), 6916–6917.
- (70) Karsten, P.; Neves, A.; Bortoluzzi, A. J.; Strähle, J.; Maichle-Mössmer, C. *Inorg. Chem. Commun.* **2002**, *5* (6), 434–438.
- (71) Jung, M.; Rentschler, E. *Zeitschrift für Anorg. und Allg. Chemie* **2015**, *641* (12–13), 2277–2281.
- (72) Barman, P.; Upadhyay, P.; Faponle, A. S.; Kumar, J.; Nag, S. S.; Kumar, D.; Sastri, C. V.; de Visser, S. P. *Angew. Chemie - Int. Ed.* **2016**, *55* (37), 11091–11095.
- (73) Vanatta, R. B.; Strouse, C. E.; Hanson, L. K.; Valentine, J. S. *J. Am. Chem. Soc.* **1987**, *109* (5), 1425–1434.
- (74) Kitajima, N.; Komatsiizaki, H.; Hikichi, S.; Osawa, M.; Moro-oka, Y. *J. Am. Chem. Soc.* **1994**, *116* (25), 11596–11597.

- (75) Singh, U. P.; Sharma, A. K.; Hikichi, S.; Komatsuzaki, H.; Moro-oka, Y.; Akita, M. *Inorganica Chim. Acta* **2006**, 359 (13), 4407–4411.
- (76) Seo, M. S.; Kim, J. Y.; Annaraj, J.; Kim, Y.; Lee, Y. M.; Kim, S. J.; Kim, J.; Nam, W. *Angew. Chemie - Int. Ed.* **2007**, 46 (3), 377–380.
- (77) Annaraj, J.; Cho, J.; Lee, Y. M.; Kim, S. Y.; Latifi, R.; De Visser, S. P.; Nam, W. *Angew. Chemie - Int. Ed.* **2009**, 48 (23), 4150–4153.
- (78) Cho, J.; Sarangi, R.; Nam, W. *Acc. Chem. Res.* **2012**, 45 (8), 1321–1330.
- (79) Kang, H.; Cho, J.; Cho, K. Bin; Nomura, T.; Ogura, T.; Nam, W. *Chem. - A Eur. J.* **2013**, 19 (42), 14119–14125.
- (80) Colmer, H. E.; Geiger, R. A.; Leto, D. F.; Wijeratne, G. B.; Day, V. W.; Jackson, T. A. *Dalton. Trans.* **2014**, 43 (48), 17949–17963.
- (81) El Ghachtouli, S.; Vincent Ching, H. Y.; Lassalle-Kaiser, B.; Guillot, R.; Leto, D. F.; Chattopadhyay, S.; Jackson, T. A.; Dorlet, P.; Anxolabéhère-Mallart, E. *Chem. Commun.* **2013**, 49 (50), 5696–5698.
- (82) Geiger, R. A.; Wijeratne, G. B.; Day, V. W.; Jackson, T. A. *Eur. J. Inorg. Chem.* **2012**, 2012 (10), 1598–1608.
- (83) Geiger, R. A.; Leto, D. F.; Chattopadhyay, S.; Dorlet, P.; Anxolabéhère-Mallart, E.; Jackson, T. A. *Inorg. Chem.* **2011**, 50 (20), 10190–10203.
- (84) Geiger, R. A.; Chattopadhyay, S.; Day, V. W.; Jackson, T. A. *J. Am. Chem. Soc.* **2010**, 132 (8), 2821–2831.
- (85) Geiger, R. A.; Chattopadhyay, S.; Day, V. W.; Jackson, T. A. *Dalton. Trans.* **2011**, 40 (8), 1707–1715.
- (86) Saravanan, N.; Sankaralingam, M.; Palaniandavar, M. *RSC Adv.* **2014**, 4 (23), 12000–12011.
- (87) Groni, S.; Blain, G.; Policar, C.; Anxolabéhère-Mallart, E. *Inorg. Chem.* **2007**, 46 (6), 1951–1953.
- (88) Leto, D. F.; Chattopadhyay, S.; Day, V. W.; Jackson, T. A. *Dalton. Trans.* **2013**, 42 (36), 13014–13025.
- (89) Groni, S.; Dorlet, P.; Blain, G.; Bourcier, S.; Guillot, R.; Anxolabéhère-Mallart, E. *Inorg. Chem.* **2008**, 47 (8), 3166–3172.
- (90) Shook, R. L.; Borovik, A. S. *Inorg. Chem.* **2010**, 49 (8), 3646–3660.
- (91) Cho, J.; Sarangi, R.; Kang, H. Y.; Lee, J. Y.; Kubo, M.; Ogura, T.; Solomon, E. I.; Nam, W. *J. Am. Chem. Soc.* **2010**, 132 (47), 16977–16986.
- (92) Shook, R. L.; Gunderson, W. A.; Greaves, J.; Ziller, J. W.; Hendrich, M. P.; Borovik, A. S. *J. Am. Chem. Soc.* **2008**, 130 (28), 8888–8889.
- (93) Coggins, M. K.; Sun, X.; Kwak, Y.; Solomon, E. I.; Rybak-Akimova, E.; Kovacs, J. A. *J. Am. Chem. Soc.* **2013**, 135 (15), 5631–5640.
- (94) Bhula, R.; Weatherburn, D. C.; Gainsford, G. J. *J. Am. Chem. Soc.* **1988**, 110 (22), 7550–7552.
- (95) Shannon, R. D. *Acta Crystallogr. Sect. A* **1976**, 32 (5), 751–767.
- (96) Jacobson, R. R.; Tyeklar, Z.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubietta, J. *J. Am. Chem. Soc.* **1988**, 110 (11), 3690–3692.
- (97) Zhang, X.; Furutachi, H.; Fujinami, S.; Nagatomo, S.; Maeda, Y.; Watanabe, Y.; Kitagawa, T.; Suzuki, M. *J. Am. Chem. Soc.* **2005**, 127 (3), 826–827.
- (98) So, H.; Park, Y. J.; Cho, K. Bin; Lee, Y. M.; Seo, M. S.; Cho, J.; Sarangi, R.; Nam, W. *J. Am. Chem. Soc.* **2014**, 136 (35), 12229–12232.
- (99) Neese, F.; Solomon, E. I. *J. Am. Chem. Soc.* **1998**, 120 (49), 12829–12848.
- (100) Jensen, K. B.; McKenzie, C. J.; Nielsen, L. P.; Pedersen, J. Z.; Svendsen, H. M. *Chem. Commun.* **1999**, No. 14, 1313–1314.
- (101) Girerd, J.-J.; Banse, F.; Simaan, A. J. *Met. Met. Species Catal. Oxidations* **2007**,

- 145–177.
- (102) Roelfes, G.; Vrajmasu, V.; Chen, K.; Ho, R. Y. N.; Rohde, J. U.; Zondervan, C.; La Crois, R. M.; Schudde, E. P.; Lutz, M.; Spek, A. L.; Hage, R.; Feringa, B. L.; Munck, E.; Que, L. *Inorg. Chem.* **2003**, *42* (8), 2639–2653.
 - (103) Groves, J. T.; Watanabe, Y.; McMurry, T. J. *J. Am. Chem. Soc.* **1983**, *105* (13), 4489–4490.
 - (104) Creager, S. E.; Murray, R. W. *Inorg. Chem.* **1987**, *26* (16), 2612–2618.
 - (105) Creager, S. E.; Raybuck, S. A.; Murray, R. W. *J. Am. Chem. Soc.* **1986**, *108* (14), 4225–4227.
 - (106) Jin, N.; Lahaye, D. E.; Groves, J. T. *Inorg. Chem.* **2010**, *49* (24), 11516–11524.
 - (107) Shook, R. L.; Peterson, S. M.; Greaves, J.; Moore, C.; Rheingold, A. L.; Borovik, A. S. *J. Am. Chem. Soc.* **2011**, *133* (15), 5810–5817.
 - (108) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, *57* (4), 918–922.
 - (109) Magherusan, A. M.; Kal, S.; Nelis, D. N.; Doyle, L. M.; Farquhar, E. R.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2019**, *58* (17), 5718–5722.
 - (110) Shin, B. K.; Kim, Y.; Kim, M.; Han, J. *Polyhedron* **2007**, *26* (15), 4557–4566.
 - (111) Mukhopadhyay, S.; Armstrong, W. H. *J. Am. Chem. Soc.* **2003**, *125* (43), 13010–13011.
 - (112) Dubois, L.; Pécaut, J.; Charlot, M. F.; Baffert, C.; Collomb, M. N.; Deronzier, A.; Latour, J. M. *Chem. - A Eur. J.* **2008**, *14* (10), 3013–3025.
 - (113) Johnston, H. M.; Freire, D. M.; Mantsorov, C.; Jamison, N.; Green, K. N. *Eur. J. Inorg. Chem.* **2022**.
 - (114) Schindler, S.; Walter, O.; Pedersen, J. Z.; Toftlund, H. *Inorganica Chim. Acta* **2000**, *303* (2), 215–219.
 - (115) Schäfer, K. O.; Bittl, R.; Zweggart, W.; Lendzian, F.; Haselhorst, G.; Weyhermüller, T.; Wieghardt, K.; Lubitz, W. *J. Am. Chem. Soc.* **1998**, *120* (50), 13104–13120.
 - (116) Yamazaki, H.; Igarashi, S.; Nagata, T.; Yagi, M. *Inorg. Chem.* **2012**, *51* (3), 1530–1539.
 - (117) Plaksin, P. M.; Stoufer, R. C.; Mathew, M.; Palenik, G. J. *J. Am. Chem. Soc.* **1972**, *94* (6), 2121–2122.
 - (118) Brewer, K. J.; Calvin, M.; Lumpkin, R. S.; Otvos, J. W.; Spree, L. O. *Inorg. Chem.* **1989**, *28* (25), 4446–4451.
 - (119) Stebler, M.; Ludi, A.; Burgi, H. B. *Inorg. Chem.* **1986**, *25* (26), 4743–4750.
 - (120) Collins, M. A.; Hodgson, D. J.; Michelsen, K.; Towle, D. K. *J. Chem. Soc. Chem. Commun.* **1987**, No. 21, 1659–1660.
 - (121) Glerup, J.; Goodson, P. A.; Hazell, A.; Hazell, R.; Hodgson, D. J.; McKenzie, C. J.; Michelsen, K.; Rychlewska, U.; Toftlund, H. *Inorg. Chem.* **1994**, *33* (18), 4105–4111.
 - (122) Baffert, C.; Collomb, M. N.; Deronzier, A.; Pécaut, J.; Limburg, J.; Crabtree, R. H.; Brudvig, G. W. *Inorg. Chem.* **2002**, *41* (6), 1404–1411.
 - (123) Hagen, K. S.; Armstrong, W. H.; Hope, H. *Inorg. Chem.* **1988**, *27* (6), 967–969.
 - (124) Towle, D. K.; Botsford, Catherine A.; Hodgson, D. J. *Inorganica Chim. Acta* **1988**, *141* (2), 167–168.
 - (125) Goodson, P. A.; Hodgson, D. J.; Glerup, J.; Michelsen, K.; Weihe, H. *Inorganica Chim. Acta* **1992**, *197* (2), 141–147.
 - (126) Limburg, J.; Vrettos, J. S.; Liable-Sands, L. M.; Rheingold, A. L.; Crabtree, R. H.; Brudvig, G. W. *Science* **1999**, *283* (5407), 1524–1527.
 - (127) Freire, D. M.; Beerli, D.; Pota, K.; Johnston, H. M.; Palacios, P.; Pierce, B. S.;

- Sherman, B. D.; Green, K. N. *Inorg. Chem. Front.* **2020**, 7 (7), 1573–1582.
- (128) Yamazaki, H.; Ueno, T.; Aiso, K.; Hirahara, M.; Aoki, T.; Nagata, T.; Igarashi, S.; Yagi, M. *Polyhedron* **2013**, 52, 455–460.
- (129) Collomb, M. N.; Deronzier, A.; Richardot, A.; Pécaut, J. *New J. Chem.* **1999**, 23 (4), 351–353.
- (130) Lessa, J. A.; Horn, A.; Bull, É. S.; Rocha, M. R.; Benassi, M.; Catharino, R. R.; Eberlin, M. N.; Casellato, A.; Noble, C. J.; Hanson, G. R.; Schenk, G.; Silva, G. C.; Antunes, O. A. C.; Fernandes, C. *Inorg. Chem.* **2009**, 48 (10), 4569–4579.
- (131) Frapart, Y. M.; Boussac, A.; Albach, R.; Anxolabéhère-Mallart, E.; Delroisse, M.; Verlhac, J. B.; Blondin, G.; Girerd, J. J.; Guilhem, J.; Cesario, M.; Rutherford, A. W.; Lexa, D. *J. Am. Chem. Soc.* **1996**, 118 (11), 2669–2678.
- (132) Ren, Y. W.; Li, J.; Zhang, F. X.; Zhang, J. H.; Guo, H. *Chinese J. Chem.* **2005**, 23 (4), 418–420.
- (133) Cooper, S. R.; Calvin, M. *J. Am. Chem. Soc.* **1977**, 99 (20), 6623–6630.
- (134) Cooper, S. R.; Dismukes, G. C.; Klein, M. P.; Calvin, M. *J. Am. Chem. Soc.* **1978**, 100 (23), 7248–7252.
- (135) Suzuki, M.; Tokura, S.; Suhara, M.; Uehara, A. *Chem. Lett.* **1988**, 17 (3), 477–480.
- (136) Horner, O.; Charlot, M.-F.; Boussac, A.; Anxolabéhère-Mallart, E.; Tchertanov, L.; Guilhem, J.; Girerd, J.-J. *Eur. J. Inorg. Chem.* **1998**, 721727.
- (137) Mahapatra, S.; Das, P.; Mukherjee, R. *Dalton. Trans.* **1993**, No. 2, 217–220.
- (138) Ramaraj, R.; Kira, A.; Kaneko, M. *Angew. Chemie Int. Ed. English* **1986**, 25 (9), 825–827.
- (139) Lee, Y.; Jackson, T. A. *ChemistrySelect* **2018**, 3 (47), 13507–13516.

Chapter 2: Generation of a Mn^{II}Mn^{III}-Peroxide and its Reactivity with Aldehydes

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2.0 Abstract

Studying the reactivity of model complexes of the active sites of enzymes offer a solution to understanding their catalytic cycles. $[\text{Mn}^{\text{II}}_2(\text{BPMP})(\text{OCCH}_3)_2](\text{ClO}_4)$ (**1**) (HBMPMP = 2,6-bis{[(bis(pyridylmethyl)amino)methyl]-4-methylphenol}) was chosen as a structural mimic of the resting state for Ib RNRs.¹ Addition KO_2 solubilised with 18-crown-6 resulted in a rapid reaction with **1** observed via UV-vis spectroscopy. **1** reacted with O_2^- to form a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**) as evidenced by UV-vis, EPR, XAS spectroscopies and mass spectrometry. Substitution of 18-crown-6 with the more efficient chelator 222-Cryptand resulted in higher yields of formation of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species **2**.

2 was found to undergo demformylation reactions with aldehydes PPA and CCA to form acetophenone and cyclohexanone respectively. The reaction between **2** and the aldehydes was monitored via UV-vis spectroscopy and revealed an exponential relationship between the rate of the reaction and the [substrate]. This was postulated to be due to a Michaelis-Menten type reaction taking place between **2** and aldehydes whereby **2** initially undergoes reversible nucleophilic attack of the aldehyde followed by an irreversible Criegee-rearrangement resulting from O-O bond scission.

2.1 Introduction

Ribonucleotide reductase (RNRs) are metalloenzymes essential to the synthesis and repair of DNA in all organisms.² RNRs catalyse the conversion of ribonucleotides to deoxyribonucleotides via the generation of a cysteinyl radical. This represented the initial and rate-limiting step in DNA synthesis and repair, making RNRs an ideal anti-cancer drug target. In light of this, understanding the mechanism of these enzymes is of the utmost importance for effective drug design.

RNRs were split into three classes (I, II, III) depending on their interaction with oxygen, however, all classes generate a cysteinyl radical to initiate ribonucleotide reduction.³ Class I RNRs are split into five subclasses (a, b, c, d, e) depending on the identity of the metal in their active site and their activating oxidant in the catalytic cycle (O_2 versus O_2^-). In its resting state the active site of Ib RNRs contained two six-coordinated Mn^{II} centres ligated by a variety of histidine and glutamate amino acids.⁴ In 2013, Stubbe and co-workers determined that in the enzyme of Ib RNRs superoxide (O_2^-) was generated upon reaction of O_2 with a nearby flavodoxin protein (Nrd_{hq}) (Figure 2.1).¹ O_2^- reacted with the Mn_2^{II} active site to form a new species that rapidly interconverted to a diamond core $Mn^{III}Mn^{IV}$ as evidenced by EPR spectrometry.¹ The intermediary preceding $Mn^{III}Mn^{IV}$ has never been trapped, and, to the best of our knowledge, no spectral evidence exists for the structure. It was postulated that the intermediate has a mixed valent hydroperoxo/peroxo $Mn^{II}Mn^{III}$ structure, which would explain the difficulty in trapping owing to this species' generally high reactivity and short lifetimes.⁵

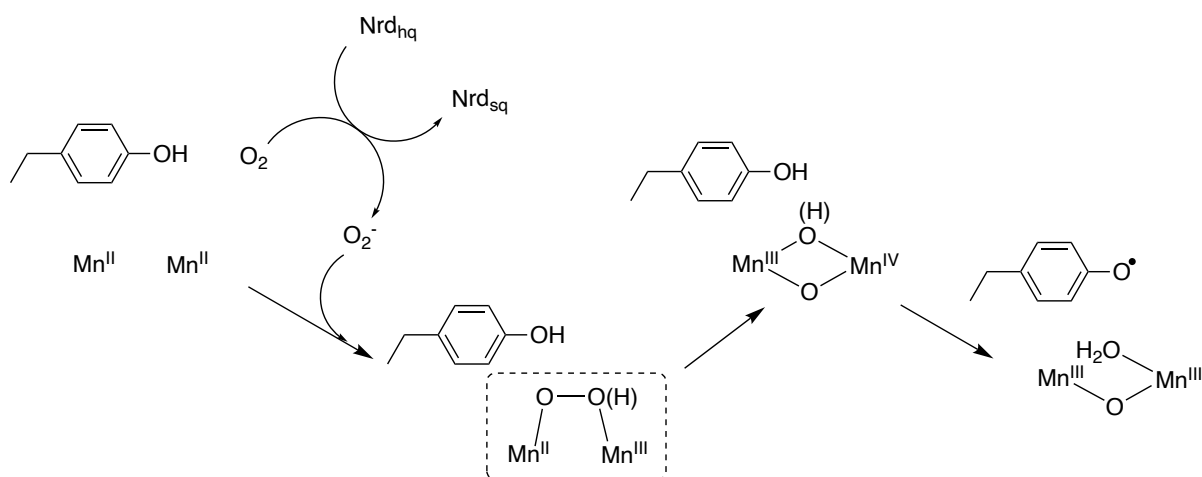


Figure 2.1: Proposed catalytic cycle in Ib RNRs.

The key intermediates in the catalytic cycle of Ib RNRs have such fleeting lifetimes that trapping and analysis of them in the native enzyme is quite difficult. One alternative approach that aims to circumvent the problems associated with studying the enzyme directly is the creation of biomimetic model complexes that imitate the enzyme's active site. Studying the reactivity of these model complexes can allow for the trapping of key intermediates and analysis of their reactivity.

For example, in 2018 McDonald and co-workers reported a Ib RNR biomimetic dinuclear manganese complex $[\text{Mn}^{\text{II}}_2(\text{O}_2\text{CCH}_3)(\text{N-Et-HPTB})](\text{ClO}_4)_2$ that reacted with superoxide (O_2^-) to form a mixed-valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species.⁶ This was the first case of experimental evidence for a peroxide intermediate following reaction with O_2^- in Ib RNRs. This new peroxide species was found to have limited reactivity, hence, we postulate that modification of the supporting ligand may increase reactivity of the system in order to learn even more about the catalytic cycle.

2.2 Formation of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -Peroxide

The complex $[\text{Mn}^{\text{II}}\text{Mn}^{\text{II}}(\text{BPMP})(\text{OCCH}_3)_2](\text{ClO}_4)$ (**1**) (HBMPMP = 2,6-bis{[(bis(pyridylmethyl)amino)methyl]-4-methylphenol}) was synthesised via previously reported procedures.^{7,8} The UV-Vis spectrum of **1** (1.5 mM) at -90°C in a 1:9 acetonitrile (MeCN)/tetrahydrofuran (THF) mixture displayed intense absorption features in the UV region from 200 - 350 nm. No other features in the visible region were observed. To **1**, a solution (250 μL) of potassium superoxide (KO_2) and 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6), releasing superoxide for reaction with **1**.⁹ **1** reacted with the formation of a new species **2**. This new species displayed two features in the UV-vis spectrum; a band at $\lambda_{\text{max}} = 440\text{ nm}$ and a lower intensity band at $\lambda_{\text{max}} = 590\text{ nm}$ (Figure 2.2, left).

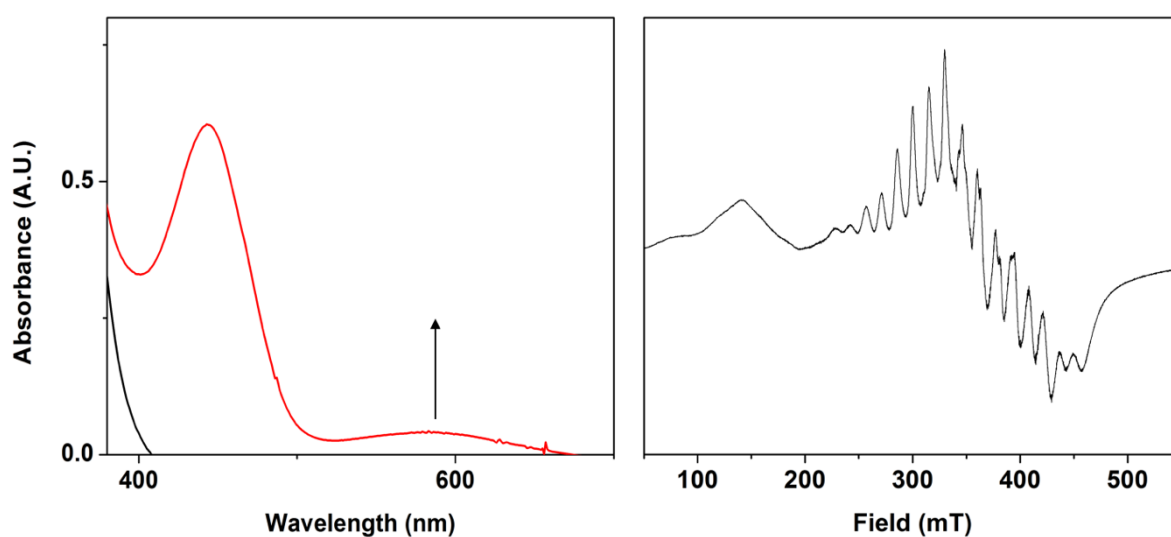


Figure 2.2: UV-vis spectra of the reaction between **1** (black trace) and KO_2 to form **2** (red trace) (left). Perpendicular X-Band spectrum of **2** measured at 2 K obtained from the reaction of **1** (1.5 mM) and KO_2 in 1:9 MeCN/THF, 9.65 GHz, 0.2 mW microwave power (right).

Due to the characteristic UV-Vis absorbance of **2**, it was postulated to be a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species. A similar $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide has been previously characterised by the McDonald group, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{O}_2(\text{N-Et-HPTB})]^{2+}$, which also showed similar features at

$\lambda_{\text{max}} = 460$ and 610 nm.¹⁰ EPR spectroscopy was also employed to determine the identity of **2**. A frozen sample was analysed via EPR at 2 K. The spectrum of **2** at 2 K revealed a 22-line signal centred at $g = 1.96$ (Figure 2.2, right). The relatively larger number of lines and g -value were indicative of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ system. Only one other $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species has been measured, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{O}_2(\text{N-Et-HPTB})]^{2+}$, synthesised by McDonald and co-workers.¹⁰ This complex displayed a 29-line signal centred at $g = 1.96$.⁴ Furthermore, the mixed valent form of **1**, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{BPMP})(\text{OCCH}_3)_2]^{2+}$ displayed a 29 line signal centred at $g \sim 2$, distinct from the 22-line signal of **2**.¹¹ This indicated **2** was a unique $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species. The broad signal located at ~ 150 mT (approximately $g = 5$) has been observed before in $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ EPR spectra and assigned as to an $S = 3/2$ spin state.⁶ Cold injection electrospray ionisation mass spectrometry (ESI-MS) was performed on a just thawed methanol solution of **2** (Figure 2.3). Analysis of the resulting spectra revealed a peak at $m/z = 770.29$ with a isotopic distribution pattern consistent with the monocation $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})(\text{ClO}_4)]^+$. Interestingly, the bridging acetate ligands of **1** were not observed in this monocation. ESI-MS studies of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{O}_2(\text{N-Et-HPTB})]^{2+}$ also revealed the absence of the bridging acetate the complex was prepared with.⁶ We speculated that during the reaction of the Mn^{II}_2 species with O_2^- the bridging acetate ligands become detached.

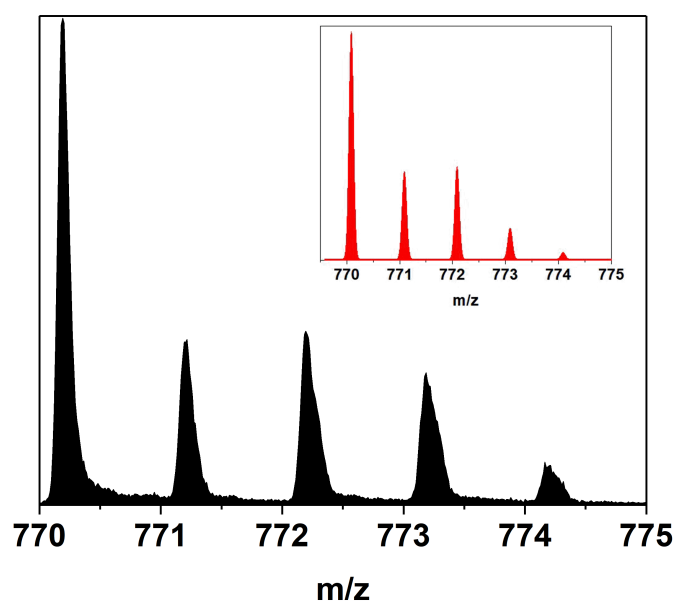


Figure 2.3: ESI-MS spectra of **2** prepared using $K^{16}O_2$. Inset: Simulated mass spectra.

Due to the transient nature of **2** and its instability at room temperature, it was not possible to grow crystals and gain insight into its structure. Instead, Mn K-edge X-ray absorption near-edge spectroscopy was performed on frozen samples of **1** and **2** to understand the oxidation states and local geometries of the metal centres (Figure 2.4). The edge energy of **1** was determined to be 6548.4 eV and the respective value for **2** was 6548.9 eV. This represented an edge energy shift of 0.5 eV upon the conversion of **1** to **2**. Typically, an increase of one in the formal oxidation of Mn is associated with an edge energy shift of 2-4 eV.^{12,13} Upon conversion of **1** to **2** an edge energy shift of 1 eV was anticipated. However, as the conversion of **1** to **2** is not quantitative, therefore a lower edge energy shift would be expected. Moreover, a common problem encountered during XANES studies of Mn complexes and enzymes is the potential for the Mn metal centres to undergo photoreduction during data collection.⁴ This may also be the source of the lower edge energy shift observed upon conversion of **1** to **2**.

The pre-edge features before the main Mn K-edge can also offer information on the oxidation state and structure of the metal centres. The pre-edge area is related to the

transitions from the 1s to the 3d orbitals.¹⁴ The pre-edge features of **1** displayed a slight doublet which is commonly associated with Mn^{II} and was assigned as the 1s transitions to the two 3d crystal field split states in a octahedral field (t_{2g} and e_g).¹⁵ The rising edge and peak of the pre-edge for both **1** and **2** were modelled with 50:50 pseudo-Voigt functions (A2.3 and A2.4). On conversion of **1** to **2** there was an increase in the pre-edge area from 8.8 to 13.5 units. An increase in pre-edge area corresponded to an decrease in the coordination number and an increase in the distortion of the geometry of the metal centre.¹² The increase in area was a result of mixing of the 3d and 4p orbitals as the Mn centres move from a octahedral to a trigonal bipyramidal geometry. The 1s→3d transition was dipole forbidden, while, the 1s→4p transition was allowed. This resulted in complexes with lower centrosymmetry, and thus increased 4p character, displaying larger pre-edge areas.¹⁶ This was consistent the conversion from the relatively symmetric initial Mn^{II} octahedral geometries to the less symmetric pentacoordinate Mn centres of **2**.

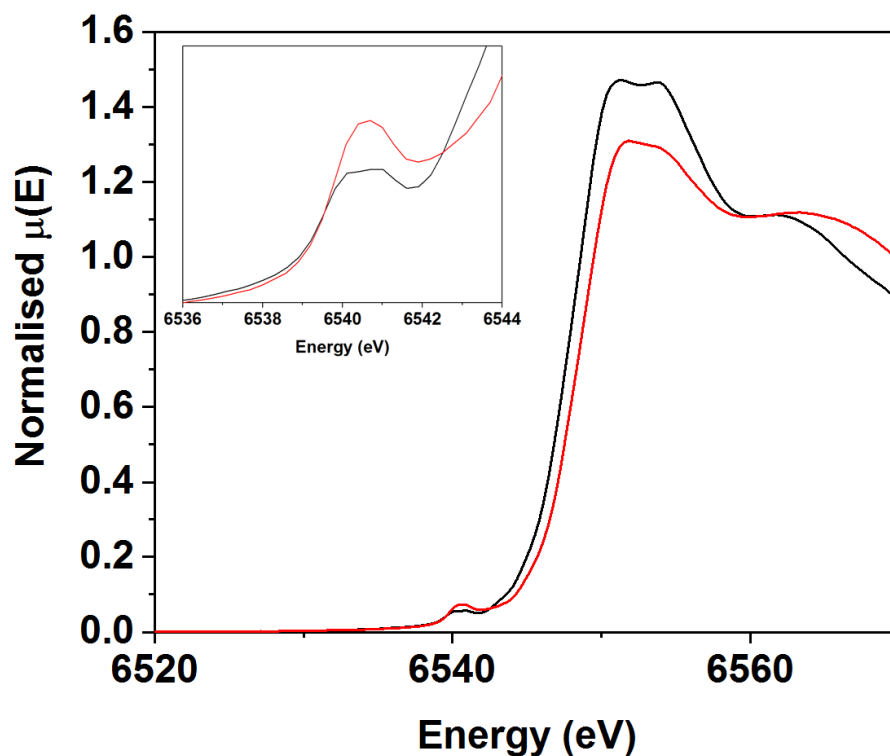


Figure 2.4: Normalised XANES spectra of **1** (black trace) and **2** (red trace). Inset: Expansion of pre-edge region.

Mn K-edge XAS was performed on Ib RNRs and the native enzyme was determined to have an edge energy of 6548.7 eV and a pre-edge peak area of 7.8 units.¹⁶ These values agreed well with those calculated for starting complex **1**, which displayed an edge energy of 6548.4 eV and pre-edge area of 8.8 units, further re-enforcing that **1** was a good mimic for the active site of Ib RNRs. This can be easily rationalised as both **1** and the active site of Ib RNRs contain two six coordinate Mn centres bridged by a bis(μ -carboxylato) moiety.^{4,17}

The combination of UV-vis, EPR, XAS spectroscopies, and mass spectrometry analysis of **2** lead us to assign the product of the reaction of **1** and O_2^- to be a $Mn^{II}Mn^{III}$ -peroxide species (Figure 2.5).

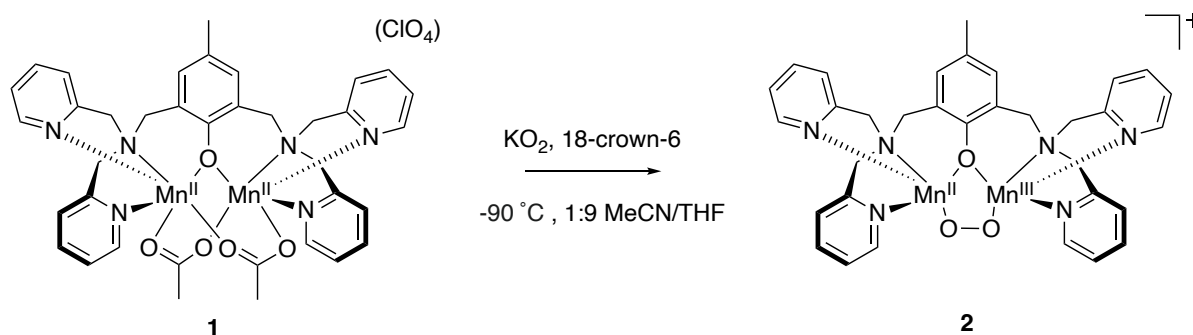


Figure 2.5: Conversion of **1** to **2** upon addition of KO_2 .

2.3 Optimisation of the Yield of **2**

It was postulated that the yield of **2** could be improved by using 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (222-Cryptand) in place of 18-crown-6, for generation of superoxide in solution. In order to compare the ability of the two macrocycles, a titration experiment was performed via UV-vis spectroscopy comparing the intensity of the $\lambda_{\text{max}} = 440$ nm feature associated with **2** when 18-crown-6 and 222-Cryptand were used to generate O_2^- (Figure 2.6).

There was a clear increase in the yield of **2** upon substitution of 18-crown-6 with the 222-Cryptand. For example, upon addition of 1 eq. of KO_2 solubilised using 18-crown-6 to **2**, the $\lambda_{\text{max}} = 440$ nm absorbance reached a maximum at 0.3 A.U. On the other hand, using 1 eq. of KO_2 solubilised with 222-Cryptand the $\lambda_{\text{max}} = 440$ nm absorbance reached a maximum at 0.85 A.U. This was more than a two-fold increase in the yield of **2**. Another advantage of the 222-Cryptand as a solubilising agent was that it allowed for higher concentration solutions of KO_2 . This was adventitious as there are smaller volumes per equivalent and the effect of dilution was lessened. Unfortunately, there was a limit of the yield of **2** as addition ≥ 2 eq. of a KO_2 /222-Cryptand solution resulted in the decay of **2**. This was clearly seen by the reduced peak-to-trough separation of the 440 nm absorbance and the loss of definition and broadening of the lower intensity 590 nm feature upon addition of 2 eq. KO_2 /222-Cryptand (Figure 2.6). The increase in yield of **2** was also

confirmed via integration of the EPR spectra and demonstrated an increase in yield of 40 % of **2** when 222-Cryptand was used instead of 18-crown-6 (A.2.5). Substitution of 18-crown-6 with 222-Cryptand afforded higher yields of **2** and therefore, 222-Cryptand was chosen to replace 18-crown-6 as the chelator for generation of **2**.

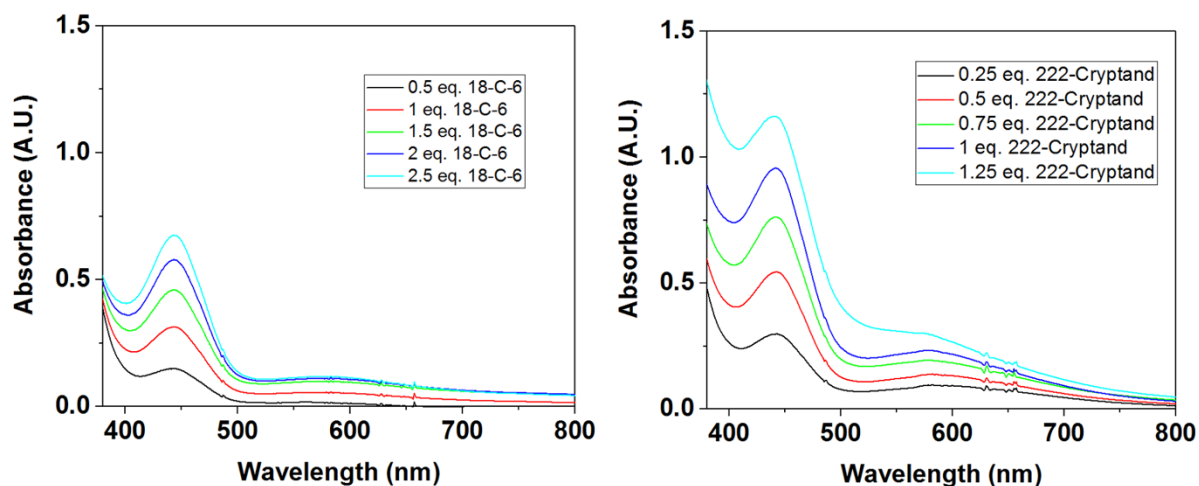


Figure 2.6: Comparison of the yields of **2** using 18-crown-6 (left) versus 222-Cryptand (right)

2.4 Nucleophilic Reactivity of **2**

The reactivity of **2** towards aldehydes substrates was investigated, in order to probe the ability of **2** to act as a nucleophile. A 1.5 mM solution of **2** in 1:9 MeCN/THF at -90 °C was reacted with the aldehyde substrates cyclohexanecarboxaldehyde (CCA) and 2-phenylpropionaldehyde (PPA) in excess. The resulting reaction was monitored via UV-vis spectroscopy. Reaction of **2** with CCA yielded a broad new absorbance feature with $\lambda_{\text{max}} = 519$ nm and a slight shoulder at $\lambda_{\text{max}} = 660$ nm (Figure 2.7). The reaction of **2** with PPA in contrast, resulted in a more defined spectrum with two new absorbance features at $\lambda_{\text{max}} = 500$ nm and $\lambda_{\text{max}} = 635$ nm (Figure 2.7). The post-reaction mixture of CCA and PPA yielded cyclohexanone and acetophenone respectively by mass spectrometry (A2.6 and A2.7).

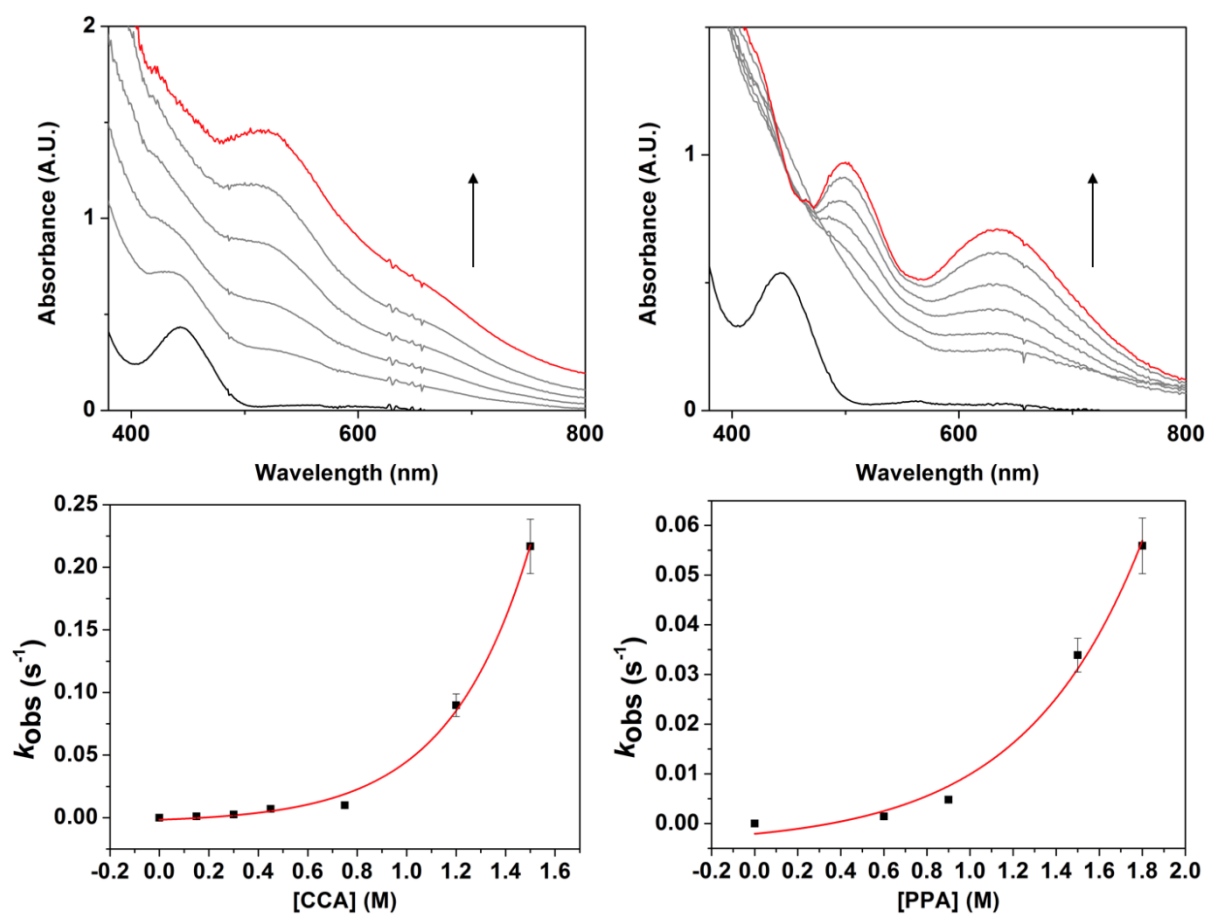


Figure 2.7: UV-vis spectra of the reaction of **2** with CCA (top left) and PPA (top right). Plot of k_{obs} versus CCA concentration determined for the reaction of **2** and CCA (bottom left). Plot of k_{obs} versus CCA concentration determined for the reaction of **2** and PPA (bottom right)

In the reaction of **2** with varying concentrations of PPA and CCA, the change in absorption at 440 nm in the UV-vis spectra was plotted versus time, and a *pseudo* first-order rate constant (k_{obs}) for the reaction was determined by fitting the resulting curve. It was found that at low aldehyde concentration, CCA and PPA displayed a linear k_{obs} relationship with respect to concentration. However, at high aldehyde concentration the k_{obs} increased exponentially (Figure 2.7). The exponential behaviour of k_{obs} was proposed to be due to initial reversible nucleophilic attack of the aldehyde by the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide. This was followed by an irreversible attack by the carbon atom α to the aldehyde carbon atom of the distal oxygen resulting in O-O bond scission, known as a Criegee-rearrangement (Figure

2.8).¹⁸ At low concentrations of aldehyde, the rate was limited by the reversible attack from the peroxide, but at higher concentrations of aldehyde the equilibrium shifts the right and the rate limiting step became the Criegee-rearrangement. Plots of k_{obs} versus [substrate] reactions with Michaelis-Menten type kinetics are expected to display saturation at higher [substrate]. Measurement of the saturation in the kinetic plot of the reaction of **2** and CCA and PPA was hindered due to the large volumes of substrate required.

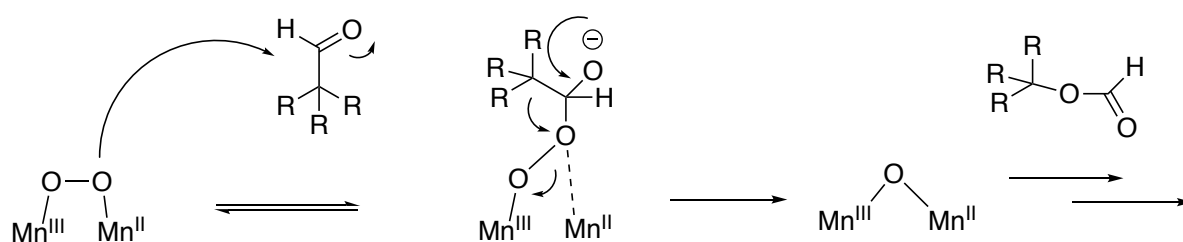


Figure 2.8: Nucleophilic attack of **2** on aldehyde substrates followed by an irreversible Criegee- rearrangement.

Previously reported mononuclear Mn^{III} -peroxide complexes have displayed similar aldehyde deformylating oxidation reactivity with PPA and CCA. In order to compare **2**'s reactivity, a second-order rate constant (k_2) value for the reaction of **2** with CCA and PPA was estimated by only fitting the linear points of the graph (A2.10 and A2.11, Table 2.3). The estimated k_2 for deformylation of PPA by **2** was an order of magnitude slower when compared to other mononuclear manganese-peroxide complexes (Table 2.3). However, it is worth noting that while the measurements of **2** were recorded at $-90\text{ }^{\circ}\text{C}$, the manganese-peroxide complexes supported by tetramethylcyclam (TMC) ligands were recorded at $20\text{ }^{\circ}\text{C}$. On the other hand, **2** displayed a k_2 rate for oxidation of CCA comparable to other TMC supported manganese-peroxides, despite the large disparity of reaction temperature ($-90\text{ }^{\circ}\text{C}$ vs $10\text{ }^{\circ}\text{C}$). **2** still presented lower CCA reactivity compared to the polypyridine supported

complexes. In light of the low-temperature conditions, **2** was considered an effective aldehyde deformylating oxidant.

Table 2.3: Aldehyde deformylation rate constants for manganese-peroxide complexes

<i>Name</i>	<i>PPA</i> $k_2 [M^{-1} s^{-1}]$ ($T[^\circ C]$)	<i>CCA</i> $k_2 [M^{-1} s^{-1}]$ ($T[^\circ C]$)	<i>Reference</i>
$[Mn^{II}Mn^{III}(O_2)(BPMP)]^+$	0.003(± 0.0008) (-90)	0.014(± 0.002) (-90)	This Work
$[Mn^{III}(O_2)(12-TMC)]^+$	0.04 (20)	0.3(± 0.2) (0)	Nam ^{19,20}
$[Mn^{III}(O_2)(14-TMC)]^+$	0.04 (20)	0.04 (10)	Nam ^{19,21}
$[Mn^{III}(O_2)(L^7py_2^{6-Me})]^+$	-	0.32 (-40)	Jackson ²²
$[Mn^{III}(O_2)(L^8py_2^H)]^+$	-	0.19(-40)	Jackson ²²

To investigate the dinuclear manganese species forming during the reaction, a frozen EPR sample of the reaction of **2** with 250 eq. of PPA was measured at 2 and 45 K (Figure 2.9). The reduction of intensity of the feature at $g = 5$ associated with high spin states of **2** indicated the consumption of the $Mn^{II}Mn^{III}$ -peroxide during the reaction. The EPR spectra **2** + 250 eq. PPA measured at 2 K revealed a sharp isotropic signal centred at $g = 2.003$. A sharp, intense signal centred at $g \sim 2$ is indicative of the formation of an organic radical during the reaction. This could indicate that **2** reacts with PPA via a radical based mechanism. This could be another explanation for the exponential rate observed at higher concentration of aldehyde due to increased radical propagation. However, radical products were not observed in the post reaction mixture. Smaller signals were present surrounding this sharp signal which was most likely manganese derived signals with much smaller intensity due to their metal-based nature. An EPR sample of the post-reaction mixture of **2** + 250 eq. PPA was also recorded at 45 K. Increasing the temperature resulted in broadening

of the signals of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, but not all dinuclear manganese species are so highly temperature-dependent. $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species, for example, have been observed at temperatures between 4 K to 140 K.^{23,24} This spectrum displayed an 11-line hyperfine feature at $g = 2.6$ and the same sharp signal at $g \sim 2$ that was present at 2 K. An 11-line signal was indicative of a equivalent Mn^{II}_2 species. The source of this signal could be unreacted starting species, **1**, or decayed intermediate **2**. Interestingly, variable temperature studies performed on **1** did not show this 11-line signal.¹¹ However, EPR spectra of **2** at 30 K revealed the same 11-line signal at $g = 2.6$ (A2.12). Therefore, we postulated that a Mn^{II}_2 side product was formed during the reaction of **1** and KO_2 . A multiline-line signal at $g \sim 1.8$ appears to also be present but it was obscured partly by the sharp isotropic line present at 2 and 45 K. This multi-line signal was metal based indicated by the lower intensity compared to the sharp $g \sim 2$ signal.

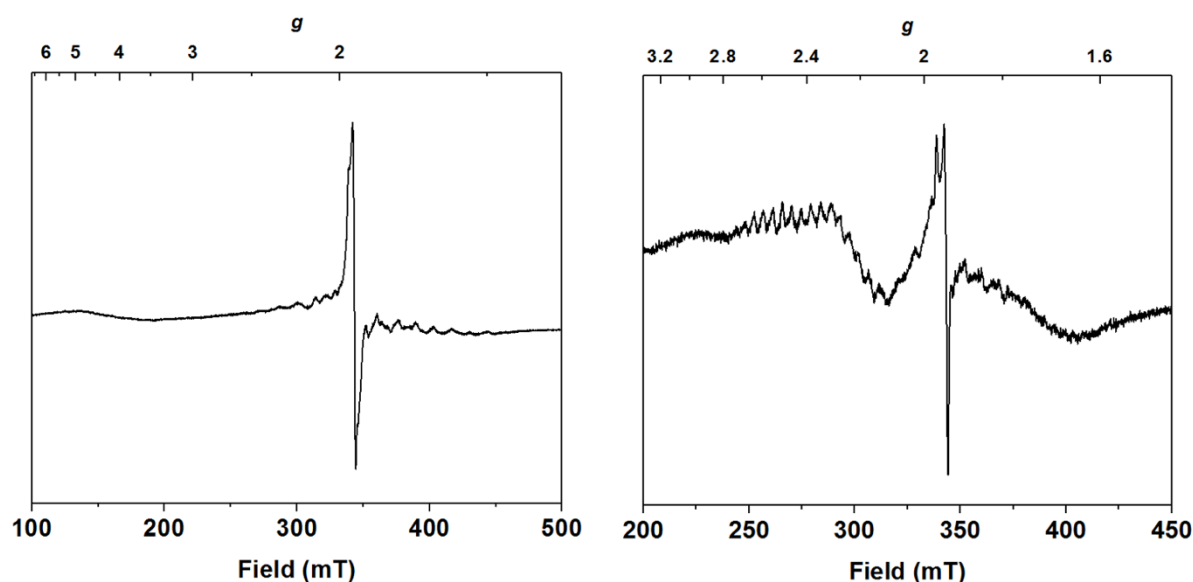


Figure 2.9: Left: Perpendicular X-band EPR spectrum of post-reaction mixture of **2** and 250 eq. of 2-phenylpropionaldehyde measure at 2 K. Right: Perpendicular X-band EPR spectrum of post-reaction mixture of **2** and 250 eq. of 2-phenylpropionaldehyde measured at 45 K (9.65 GHz, 0.2 mW microwave power).

2.5 Conclusions

It was observed complex $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{BPMP})(\text{OCCH}_3)_2](\text{ClO}_4)$ (**1**) reacted with superoxide to form a new species **2** via UV-vis spectroscopy. **2** was identified as a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide using EPR spectroscopy, and displayed a 22-line signal centred at $g = 1.96$. The reactivity of **2** was investigated with aldehydes. **2** was found to be an deformlyating oxidant in the reaction with the aldehydes PPA and CCA. **2** displayed an exponential k_{obs} trend with respect to aldehyde concentration. It was postulated the source the exponential trend was due to initial reversible nucleophilic attack of the aldehyde by the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide followed a Criegee rearrangement. EPR spectroscopy of the post-reaction mixture of **2** + 250 eq. of PPA revealed a sharp signal at $g = 2$, characteristic of an organic radical signal. This sharp signal partially masked less-intense metal-based signals present in the spectra.

2.6 Experimental

Materials

All reactions with air-sensitive materials were performed in a glove box in a N₂ atmosphere or under inert conditions using inert Schlenk line techniques. All reagents and solvents were purchased from commercial sources. Anhydrous *N,N*-dimethylformamide (DMF) was purchased and used without further purification. Anhydrous tetrahydrofuran (THF), acetonitrile (MeCN) were dispensed through an MBraun MB-SPS-5 Solvent Purification System and deoxygenated by purging with argon.

N,N-bis(2-picoyl)amine and 2,6-bis(chloromethyl)-4-methylphenol were synthesised as previously described.^{25,26} The ligand 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol (HBPMP) was synthesised as previously reported.⁷ The synthesis of [Mn₂(O₂CCH₃)₂(BPMP)](ClO₄), **1**, was carried out as previously reported.²⁷

Caution: KO₂ and perchlorate salts of metal complexes are potentially explosive and must be handled with care and in small quantities. No problems were encountered during this project.

Physical Methods

¹H NMR analysis was performed on a Bruker Avance III 400 MHz instrument. Electrospray ionisation mass spectra (ESI-MS) were obtained using micromass time of flight spectrometer (TOF), interfaced to a Waters 2690 HPLC, or by direct injection in the mass spectrometry instrument. Electronic absorption spectra were recorded in quartz cuvettes on a Hewlett Packard (Agilent) 8453 diode array spectrophotometer (190 – 1100 nm range) coupled to a liquid nitrogen cooled cryostat from Unisoku Scientific Instruments (Osaka, Japan). Electronic paramagnetic resonance (EPR) measurements were conducted

on a Bruker Eleksys E-500 spectrometer with an Oxford ESR 910 liquid helium cryostat and an Oxford temperature controller.

X-ray absorption spectroscopy methods: The Mn-K-edge X-ray absorption data were collected on beamline 7-3 of the Stanford Synchrotron Radiation Lightsource (SLAC National Accelerator Lab, Menlo Park, CA, USA). Data were collected with the storage ring operating at 3.0 GeV and 500 mA, using a LN2 cooled Si(220), $\phi = 90^\circ$ double-crystal monochromator, calibrated by using the first inflection point of a Mn foil (6539.0 eV). The monochromator was detuned by $\sim 50\%$ for higher harmonic rejection. A Canberra 32-element solid state germanium detector was used for fluorescence detection. All the measurements were performed at ambient pressure at ~ 17 K using an Oxford helium cryostat that was cooled by closed-cycle He gas loop. The parameters used for the scans were the following: 10 eV steps/1 second integration time in the pre-edge region, 0.3 eV steps /2 second integration time in the edge, and 0.05k steps in the EXAFS, with integration time increasing in a k^2 -weighted fashion from 2 to 4 seconds over the energy range ($k_{\text{max}} = 12.1k$). The total detector counts were typically 3-7k, well within the linear range of the detector electronics. Each sample was monitored for photoreduction. Complex **2** was found to be photoreduced by the X-rays and so only one scan was obtained per spot. Delrin ® Mossbauer/XAS cups with sample windows of 4 mm x 10 mm. Elaboration of the XAS data, including averaging, background removal and normalization, was performed using Athena.²⁸ Edge energies were obtained by taking the first derivative of the rising edge, while pre-edge energies were identified using the second derivative. Peak fitting analysis employed the program Fityk,²⁹ using the protocol developed by Westre et al. for analysing Fe K-edge pre-edge peaks.¹⁴ Briefly, a 15-18 eV segment of the normalized $\mu(E)$ spectrum was fit around the pre-edge, with pre-edge peaks modelled using pseudo-Voigt functions having a fixed 50:50 mixture of Gaussian: Lorentzian character, while the background

absorption was modelled with a single pseudo-Voigt function where the proportion of Gaussian: Lorentzian character was allowed to float. Four slightly different energy ranges were fit separately, and the results averaged to generate the reported peak fits.

Experimental Procedures

General procedure for the reaction of 1 with KO₂ and 18-Crown-6

A 1:9 CH₃CN/THF solution (1.5 mM) of complex **1** was prepared. In a quartz cuvette 2 mL of a 1.5 mM solution of complex **1** was cooled to -90 °C. KO₂ (0.0056 g, 0.01 M) and 18-crown-6 ether (0.079g, 50 mM) in 7.5 ml of DMF was added to the solution of **1** (250 μL). The reaction progress was monitored using electronic absorption spectroscopy. Lower yields were observed (less intense absorption features) with high equivalencies of KO₂ and at higher concentrations of **1**.

General procedure for the reaction of 1 with KO₂ and 222-Cryptand

A 1:9 CH₃CN/THF solution (1.5 mM) of complex **1** was prepared. In a quartz cuvette 2 mL of a 1.5 mM solution of complex **1** was cooled to -90 °C. KO₂ (0.0056 g, 0.04 M) and 222-Cryptand (0.06 g, 0.08 M) were dissolved in DMF (2 mL). 75 μL of this solution was added to the solution containing **1**. The reaction progress was monitored using electronic absorption spectroscopy. Lower yields were observed (less intense absorption features) with high equivalencies of KO₂ and at higher concentrations of **1**.

General procedure for the reaction of 2 with substrates

A solution of **2** in 1:9 MeCN/THF at -90 °C was prepared using the above procedure. Substrates were added as concentrated solutions in DMF or 1:9 MeCN/THF to a solution of **2** at -90 °C. The reactions were monitored using electronic absorption spectroscopy.

Frozen EPR Sample Preparation

The samples for EPR analysis were prepared by transferring ~ 1 mL of the desired solution from the quartz cuvette, pre-cooled to -90 °C, via a pre-cooled pipette to a pre-cooled EPR tube and immediately freezing in liquid nitrogen. EPR spectra were either recorded at 77 K (9.21 GHz, 2 mW microwave power and a 0.5 mT field modulation amplitude), 45 K (9.64 GHz, 0.2 mW microwave power and a 1 mT field modulation amplitude), or 2 K (9.65 GHz, 0.2 mW microwave power and a 1 mT field modulation amplitude).

Frozen ESI-MS Sample Preparation

The samples for frozen MS analysis were prepared by transferring ~ 0.1 mL of the desired solution from the quartz cuvette, pre-cooled to -90 °C, via a pre-cooled MS sample vial with pre-cooled methanol and immediately freezing in liquid nitrogen. ESI- MS was performed on the just cooled sample.

2.7 References

- (1) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, *135* (10), 4027–4039.
- (2) Eriksson, M.; Jordan, A.; Eklund, H. *Biochemistry* **1998**, *37* (38), 13359–13369.
- (3) Cotruvo, J. A.; Stubbe, J. *Annu. Rev. Biochem.* **2011**, *80* (1), 733–767.
- (4) Boal, A. K.; Cotruvo, J. A.; Stubbe, J.; Rosenzweig, A. C. *Science* **2010**, *329* (5998), 1526–1530.
- (5) Shook, R. L.; Gunderson, W. A.; Greaves, J.; Ziller, J. W.; Hendrich, M. P.; Borovik, A. S. *J. Am. Chem. Soc.* **2008**, *130* (28), 8888–8889.
- (6) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, *57* (4), 918–922.
- (7) Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, *39* (16), 3526–3536.

- (8) Blanchard, S.; Blondin, G.; Rivière, E.; Nierlich, M.; Girerd, J. J. *Inorg. Chem.* **2003**, *42* (15), 4568–4578.
- (9) Kishioka, S. Y.; Takekawa, T.; Yamada, A. *Anal. Sci.* **2004**, *20* (10), 1465–1466.
- (10) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, *57* (4), 918–922.
- (11) Diril, H.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S.; Chang, H. R.; Nilges, M. J.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, *111* (14), 5102–5114.
- (12) Schreiber, R. E.; Cohen, H.; Leitus, G.; Wolf, S. G.; Zhou, A.; Que, L.; Neumann, R. *J. Am. Chem. Soc.* **2015**, *137* (27), 8738–8748.
- (13) Weng, T. C.; Hsieh, W. Y.; Uffelman, E. S.; Gordon-Wylie, S. W.; Collins, T. J.; Pecoraro, V. L.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **2004**, *126* (26), 8070–8071.
- (14) Westre, T. E.; Kennepohl, P.; DeWitt, J. G.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1997**, *119* (27), 6297–6314.
- (15) Farges, F. *Phys. Rev. B - Condens. Matter Mater. Phys.* **2005**, *71* (15), 155109.
- (16) Stemmler, T. L.; Sossong, T. M.; Goldstein, J. I.; Ash, D. E.; Elgren, T. E.; Kurtz, D. M.; Penner-Hahn, J. E. *Biochemistry* **1997**, *36* (32), 9847–9858.
- (17) Blanchard, S.; Blondin, G.; Rivière, E.; Nierlich, M.; Girerd, J.-J. *Inorg. Chem.* **2007**, *42* (15), 4568–4578.
- (18) Pirovano, P.; Magherusan, A. M.; McGlynn, C.; Ure, A.; Lynes, A.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2014**, *53* (23), 5946–5950.
- (19) Kang, H.; Cho, J.; Cho, K. Bin; Nomura, T.; Ogura, T.; Nam, W. *Chem. - A Eur. J.* **2013**, *19* (42), 14119–14125.
- (20) Seo, M. S.; Kim, J. Y.; Annaraj, J.; Kim, Y.; Lee, Y. M.; Kim, S. J.; Kim, J.; Nam, W. *Angew. Chemie - Int. Ed.* **2007**, *46* (3), 377–380.
- (21) Annaraj, J.; Cho, J.; Lee, Y. M.; Kim, S. Y.; Latifi, R.; De Visser, S. P.; Nam, W. *Angew. Chemie - Int. Ed.* **2009**, *48* (23), 4150–4153.
- (22) Geiger, R. A.; Chattopadhyay, S.; Day, V. W.; Jackson, T. A. *Dalton. Trans.* **2011**, *40* (8), 1707–1715.
- (23) Goodson, P. A.; Hodgson, D. J.; Glerup, J.; Michelsen, K.; Pedersen, E. *Inorg. Chem.* **1990**, *29* (3), 503–508.
- (24) Lessa, J. A.; Horn, A.; Bull, É. S.; Rocha, M. R.; Benassi, M.; Catharino, R. R.; Eberlin, M. N.; Casellato, A.; Noble, C. J.; Hanson, G. R.; Schenk, G.; Silva, G. C.; Antunes, O. A. C.; Fernandes, C. *Inorg. Chem.* **2009**, *48* (10), 4569–4579.
- (25) Borovik, A. S.; Que, L.; Papaefthymiou, V.; Taylor, L. F.; Anderson, O. P. *J. Am. Chem. Soc.* **1989**, *111* (16), 6183–6195.
- (26) Raycroft, M. A. R.; Maxwell, C. I.; Oldham, R. A. A.; Andrea, A. S.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2012**, *51* (19), 10325–10333.

- (27) Suzuki, M.; Mikuriya, M.; Murata, S.; Uehara, A.; Oshio, H.; Kida, S.; Saito, K. *Bull. Chem. Soc. Jpn.* **1987**, *60* (12), 4305–4312.
- (28) Ravel, B.; Newville, M. *J. Synchrotron Radiat.* **2005**, *12* (4), 537–541.
- (29) Wojdyr, M. *J. Appl. Crystallogr.* **2010**, *43* (5), 1126–1128.

Chapter 3: Acid Activation of a Mn^{II}Mn^{III}-Peroxide with Relevance to the Catalytic Cycle of Ib RNRs.

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3.0 Abstract

Ribonucleotide reductase (RNR) are metalloenzymes that contain the unique ability to manufacture deoxyribonucleotides, the building blocks for DNA synthesis in all organisms. Understanding the mechanism of how these enzymes function would provide invaluable insight into the development of anti-cancer drugs. Unfortunately, the complete catalytic cycle of class Ib RNRs, a subclass of the enzyme, is still unknown due to the short lifetimes of key intermediates making them difficult to trap and analyse. Model complexes of the active sites of RNRs offer the ability to isolate and study these intermediates to garner key information about their mechanism.

$[(\text{Mn}^{\text{II}})_2(\text{BPMP})(\text{OAc})_2(\text{ClO}_4)]$ (**1**), was reacted with the activating oxidant in the cycle of class Ib RNRs, superoxide (O_2^-), resulting in the rapid formation of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**) species that was confirmed using EPR, X-ray absorption, UV-vis spectroscopies and mass spectrometry. **2** was found to be relatively unreactive, however, addition of pTsOH resulted in the generation of a new species (**4**). **4** was confirmed to be a $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex via UV-vis, EPR, and X-ray absorption spectroscopies and mass spectrometry analysis. Remarkably, **4** displayed similar EPR spectra to the species formed in the catalytic cycle of Ib RNRs preceding oxidation, implying that **4** was a good mimic for the enzyme. **4** was capable of reaction with a variety of O-H bond containing substrates. EPR analysis of the post-reaction mixtures determined that the corresponding oxygen based radical species were generated after reaction with **4**, the same reactivity observed in Ib RNRs. Kinetic analysis of the reaction of **4** with substrates determined that the oxidation occurred through a hydrogen atom transfer mechanism. This was the first example of experimental insight into the method of oxidation employed by Ib RNRs.

3.1 Introduction

Ribonucleotide reductase (RNR) are a class of enzymes responsible for catalysing the conversion of ribonucleotides to the corresponding deoxyribonucleotide, providing precursors for DNA synthesis and repair in all organisms.¹ RNRs are an important target for anti-cancer drugs, therefore, understanding the enzymes' mechanism is of the utmost importance.² There are three classes of RNRs (I, II, and III) and each initiates the ribonucleotide reduction upon generation of a cysteinyl radical.³ What differentiates each class was their method of generation of the cysteinyl radical.³ Class I is further divided into five subclasses (a, b, c, d, and e) depending on the identity of their metallo-cofactors.⁴ Class Ib RNRs utilised superoxide, (O_2^- , Figure 3.1), which was generated upon reduction of O_2 by a nearby flavodoxin protein (NrdI).

O_2^- reacted with a Mn^{II}_2 cofactor and was postulated to form a mixed valent $Mn^{II}Mn^{III}$ -peroxide which undergoes O-O bond cleavage and generated a $Mn^{III}(\mu-OH)(\mu-O)Mn^{IV}$ species, supported by the measurement of a characteristic 16-line electron paramagnetic resonance (EPR) signal.⁵ This $Mn^{III}Mn^{IV}$ was postulated to be the active species responsible for oxidation of a tyrosine to a tyrosyl radical. Measurement of the intermediate species following O_2^- reaction and preceding $Mn^{III}Mn^{IV}$ formation have thus far been unsuccessful as detection of Mn_2 -peroxide species are extremely difficult due to their low-stability and high reactivity.⁶ Questions also linger about the possible role of a proton in the mechanism of Ib RNRs. One possibility was that superoxide reacted with the Mn^{II}_2 cluster in its protonated form (HO_2^-) to form a $Mn^{II}Mn^{III}$ -OO(H) adduct.⁵

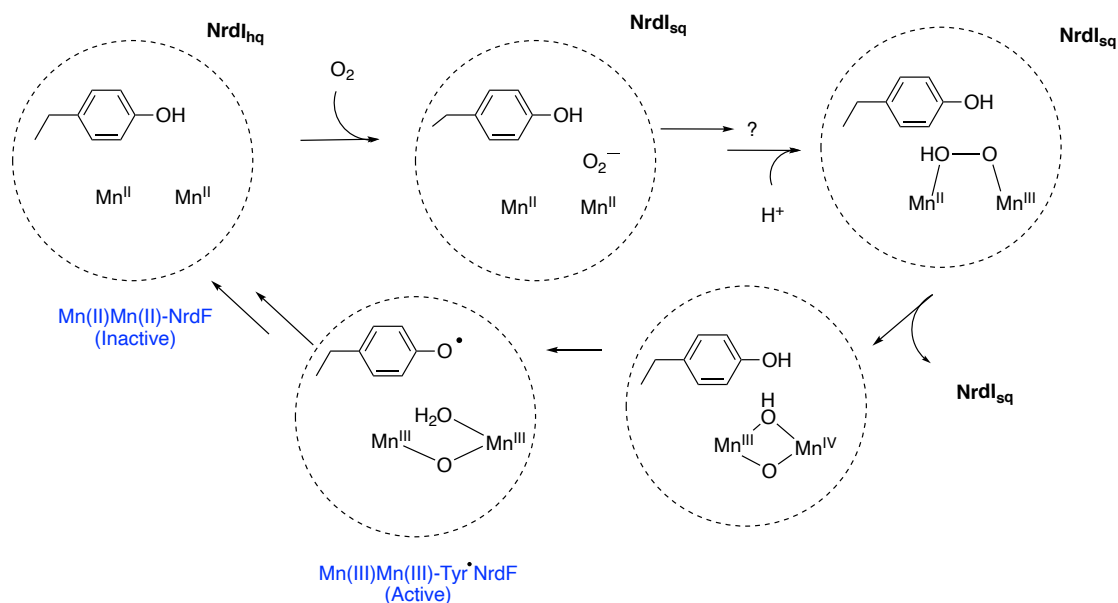


Figure 3.1: Proposed mechanism for Class I b RNRs.

Another area of uncertainty surrounding the catalytic cycle of Ib RNRs was the mechanism of generation of the tyrosyl radical by the $Mn^{III}Mn^{IV}$ species and if it employed stepwise or concerted proton coupled electron transfer (PCET) (Figure 3.2). The reaction may proceed in a single-step concerted proton-electron transfer step, also known as hydrogen atom transfer (HAT). The other option was a stepwise process whereby electron transfer (ET) was followed by proton transfer (PT) or vice versa. It was postulated that the tyrosyl oxidation would proceed via a HAT mechanism as this would avoid the formation of high-energy intermediates that could damage the enzymes structure.⁷ While the mechanism of movement of the radical from tyrosyl to cysteinyl has been extensively studied in Ib RNRs, little experimental evidence has been provided for mechanism of initial tyrosine oxidation.⁸

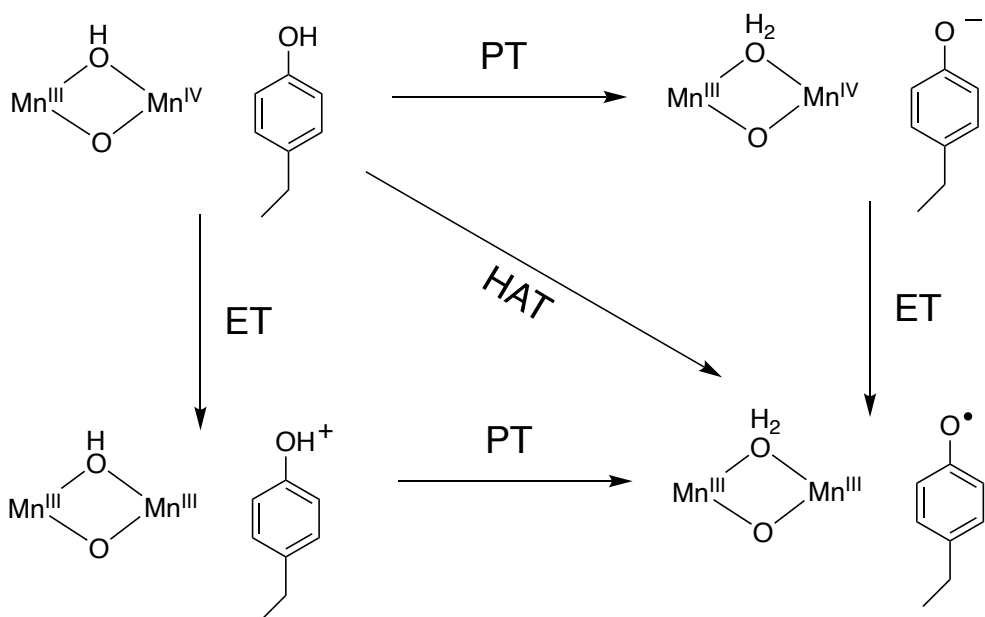


Figure 3.2: Reaction diagram showing the pathway of stepwise proton transfer (PT) electron transfer (ET) and stepwise ETPT (edges) and the concerted PTET mechanism (HAT) of possible mechanisms of the tyrosyl radical formation.

An alternative method for understanding the mechanism of enzymes was using small molecule mimics of active sites and probing their reactivity. Over the years several complexes have been synthesised, relevant to the cycle of class Ib RNRs. $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes in particular have been studied in depth due to their relative stability compared to Mn-peroxide complexes.⁹ Recently, a Mn^{II}_2 complex, $[(\text{Mn}^{\text{II}}_2)(\text{BPMP})(\text{OAc})_2(\text{ClO}_4)]$, was reported to react with O_2^- to form a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species.¹⁰ The post oxidation EPR spectrum displayed a 22-line signal centred at $g = 1.96$, consistent with a mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ system. This $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide represented a promising mimic of the species formed after oxidation of the Mn^{II}_2 cluster with O_2^- and studying the reactivity of this species may lead to insight into the intermediates and mechanism of oxidation in this enzyme.

3.2 Results and Discussion

$[\text{Mn}^{\text{II}}_2(\text{BPMP})(\text{OCCH}_3)_2(\text{ClO}_4)]$, (**1**, HBPMP = 2,6-bis[bis(2-pyridylmethyl)amino)methyl]-4-methylphenol) was prepared according to previous reports.¹⁰ The previously reported $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ (**2**) was prepared using a modified procedure.¹¹ **1** (1.5 mM) was reacted with KO_2 (1 equiv.) in 1:9 CH_3CN /tetrahydrofuran (THF) at -90°C to yield **2** (Figure 3.3). However, the previously used 18-crown-6 (used to solubilise KO_2) was replaced with 222-Cryptand (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) as it afforded an increase in yield of approximately 40% of **2** (A3.13). The substitution of 222-Cryptand for 18-crown-6 did not have an effect on the spectroscopic properties of **2**, as evidenced by the same electronic absorption features ($\lambda_{\text{max}} = 440, 590 \text{ nm}$, A3.1).¹⁰ EPR spectroscopy analysis of **2** displayed a 22-line signal centred at $g = 2$, observed previously in the preparation of **2** (Chapter 2).¹⁰ Cold injection electrospray mass spectrometry (ESI-MS) on a just-thawed solution of **2** displayed a peak at $m/z = 770.29$ consistent with the isotope distribution pattern of the monocation $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{-O}_2(\text{BPMP})(\text{ClO}_4)]^+$. **2** was capable of performing deformylation of aldehydes, however, towards substrates containing X-H bonds **2** was found to be unreactive.¹⁰

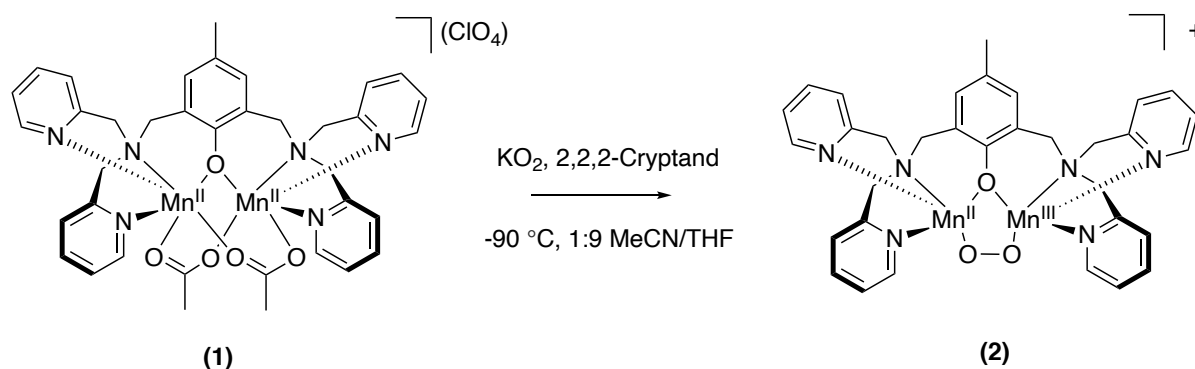


Figure 3.3: Reaction of **1** with superoxide to generate a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species, **2**.

3.3 Strong Acid Investigation

Addition of the aqueous hydrochloric acid (HCl) to a solution of **2** (generated from **1** (1.5 mM) in 1:9 CH₃CN/THF) at -90°C resulted in an immediate reaction as evidenced by the formation of three new bands in the electronic absorption spectrum (Figure 3.4, $\lambda_{\text{max}} = 445, 555, 710 \text{ nm}$). This was indicative of the formation of a new species (**3**). The new features reached a maximum after 3 equiv. of HCl were added to **2**. Addition of >3 equiv. of HCl resulted in precipitation (A3.3). The features of new species **3** were similar to those assigned to the mixed-valent form of the complex, [Mn^{II}Mn^{III}(BPMP)(OAc)₂](ClO₄)₂, previously characterised by Hendrickson and co-workers ($\lambda_{\text{max}} = 427, 478, \text{ and } 620 \text{ nm}$).¹² We postulated that in the reaction of strong acid species with **2** a new species, **3**, was formed and the shift to lower energy was possibly due to chloride ion (derived from HCl) coordination to the manganese centres, rather than OCCH₃. We tentatively assigned the species obtained from the reaction between **2** and the strong acid HCl to be a new Mn^{II}Mn^{III} species, that no longer possessed a peroxide ligand.

EPR spectroscopy was employed in order to further analyse the identity of the new species (**3**) formed during the reaction of **2** + HCl. A frozen solution of **2** + HCl measured at 77 K revealed a 6-line signal centred at $g = 2$ (Figure 3.4, inset). No other signals were observed in the EPR spectrum. The number of hyperfine lines was consistent with the formation of mononuclear Mn^{II} ions $\{(2nI + 1) = (2 \cdot 1 \cdot 5/2 + 1) = 6\}$, where n = number of atoms and I = Mn⁵⁵ nuclear spin}. Moreover the average hyperfine coupling constant (A_{av}) for the six-line signal was determined to be 9.6 mT, which agreed with the values reported for free mononuclear Mn^{II} ions.^{13–15} The free Mn^{II} ions exhibited an intense EPR signal and therefore obscured any potential dinuclear Mn EPR signals and prevented assignment of the oxidation states of **3**. Addition of strong acids to **2** may have resulted in the

demetallation of the complex generating free Mn^{II} ions, however, the reaction mechanism is still unclear.

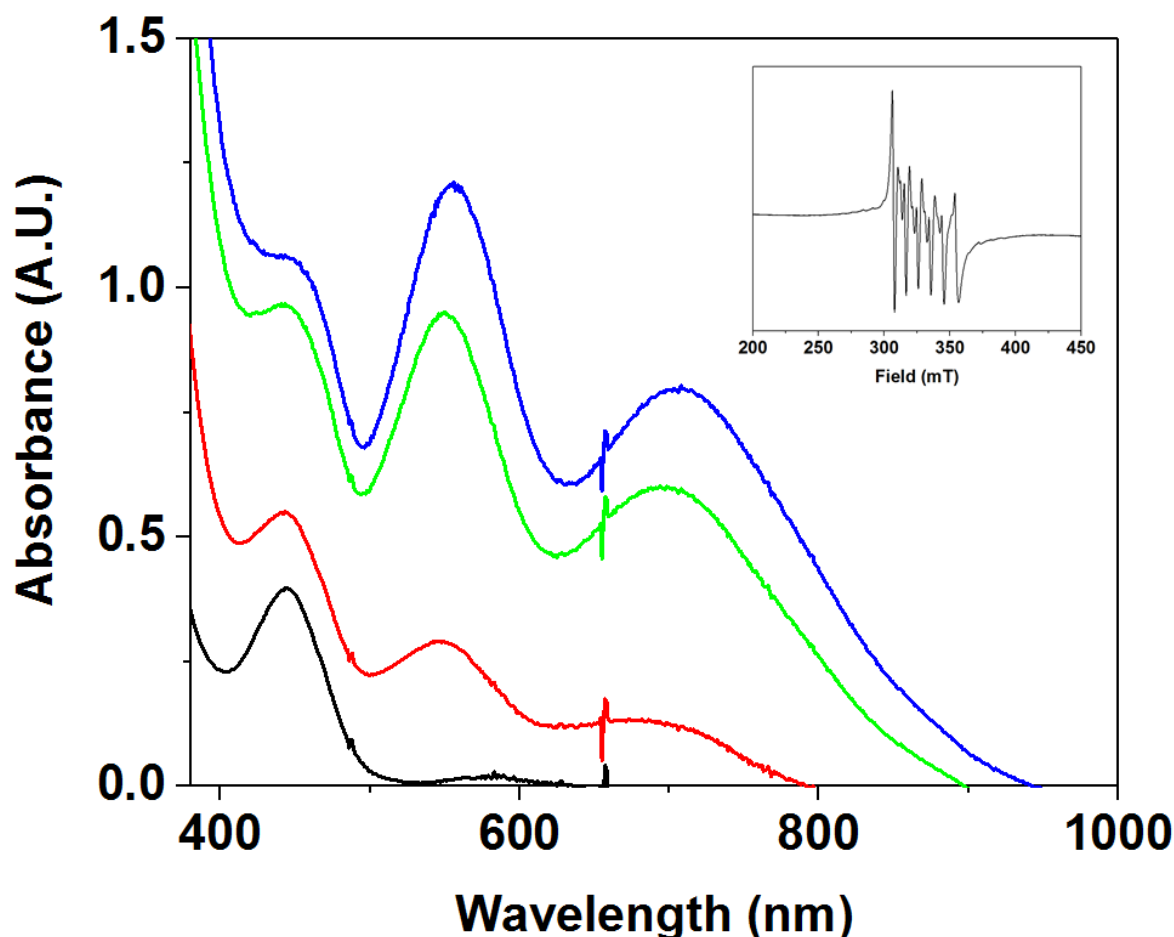


Figure 3.4: Electronic absorption features of the reaction of **2** (black trace) with 1 eq (red trace), 2 eq. (green trace), and 3 eq. (blue trace) of HCl at $-90\text{ }^{\circ}\text{C}$ monitored by UV-Vis spectroscopy. Inset: EPR spectrum of the post-reaction mixture of **2** + 3 eq. HCl measured at 77K (9.65 GHz, 0.2 mW microwave power)

3.4 Weak Acid Investigation

A 1.5 mM solution of **2** in 1:9 MeCN/THF at $-90\text{ }^{\circ}\text{C}$ was reacted with *para*-toluenesulfonic acid (pTsOH) and monitored via UV-vis spectroscopy (Figure 3.5). Addition of pTsOH resulted in the immediate formation of new electronic absorption features associated with a new species (**4**). The maximum yield of **4** was achieved upon addition of 2 eq. of acid, and displayed one intense band at $\lambda_{\text{max}} = 550\text{ nm}$ that trailed into the near-IR region. **4** was found to be a metastable species with $t_{1/2} = 1225\text{ seconds}$ at $-90\text{ }^{\circ}\text{C}$. The electronic

absorption features of **4** were red-shifted in comparison to that of the Mn^{II}Mn^{III}-peroxide of **2**. This was characteristic of diamond core bis(μ -oxo)Mn^{III}Mn^{IV} species which typically showed features between 500 and 700 nm.¹⁶ For example, the complex [bis(μ -oxo)Mn^{III}Mn^{IV}(N4py)₂]³⁺ reported by Jackson *et al.* displayed an intense feature at λ_{max} = 565 nm and an less intense feature at λ_{max} = 667 nm.¹⁷

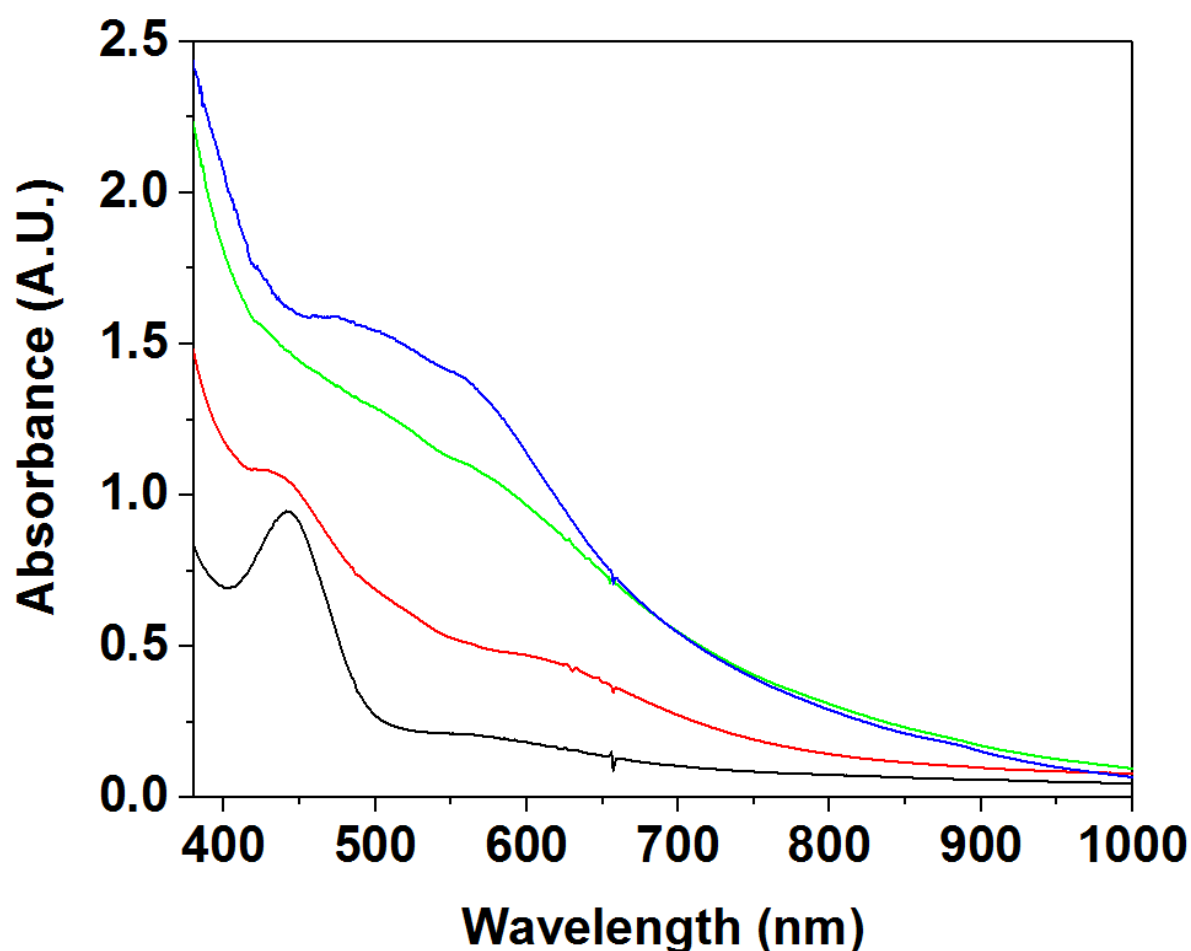


Figure 3.5: Electronic absorption spectra of **2** (black) + 1 eq. (red trace), 1.5 eq. (green trace), and 2 eq. of pTsOH (**4**, blue trace).

EPR studies were performed on the post-reaction mixtures of **2** + 1, 1.5, and 2 eq. of pTsOH at 2 K in order to understand what new species were forming in the reaction (Figure 3.6). Upon addition of 1 eq. of pTsOH to **2**, there was formation of a new 8-line signal centred at $g = 2$ which we postulated to be the new species **4** observed in UV-vis spectroscopy

(Figure 3.6, red trace), however, residual $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide from unreacted **2** remained in the solution and possibly obscured some of the new signal, preventing assignment of the new species. Addition of 1.5 eq. of pTsOH to **2** revealed more of **4**'s signal centred at $g = 2$ (Figure 3.6, green trace), but unreacted **2** still prevented accurate assignment of this new signal. The EPR spectra of **2** + 2 pTsOH displayed the clearest view of **4**'s signal and showed 10 lines centred at $g = 2$ (Figure 3.6, blue trace). Unfortunately, some unreacted **2** was still visible around the edges of the new signal. Addition of 3 eq. pTsOH was attempted in order to consume all of the unreacted $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, however, this resulted in lower intensity of **4** in the EPR spectrum (A3.11). We postulate that >2 eq. of pTsOH caused the decay of **4** and resulted in lower yields.

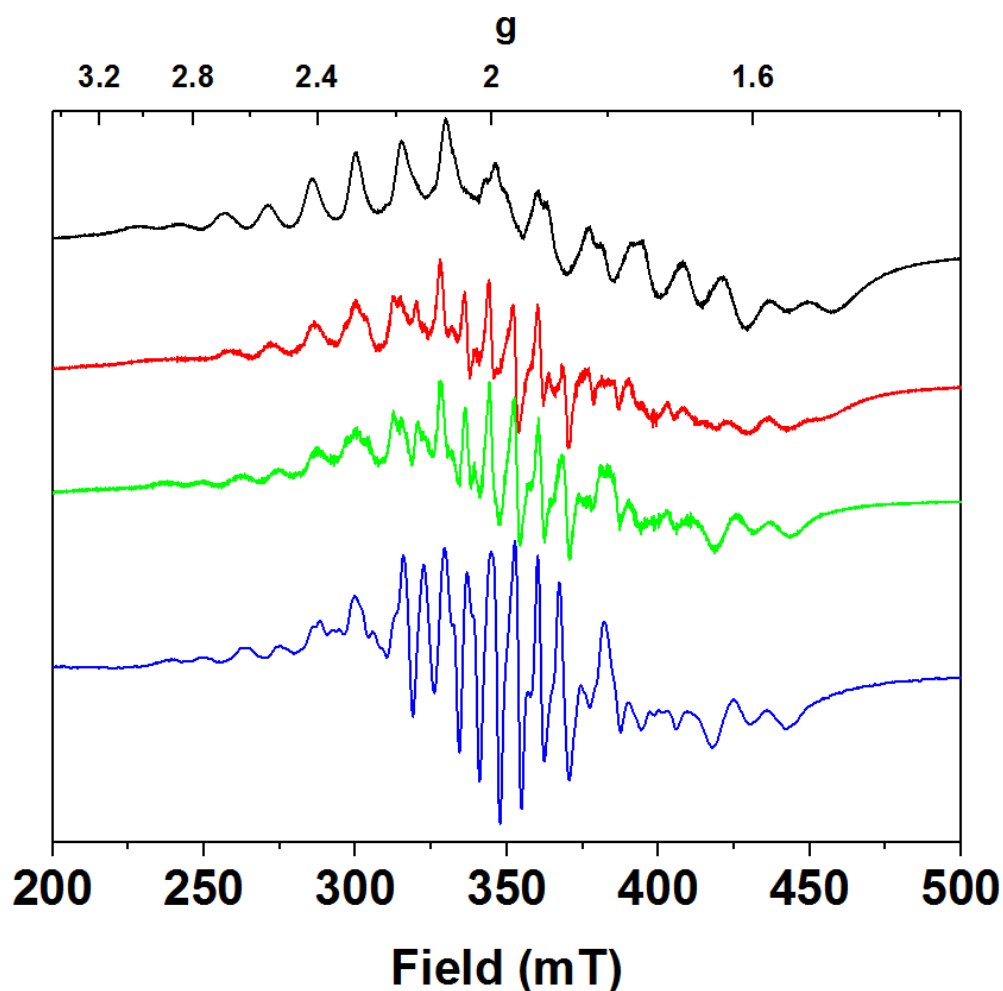


*Figure 3.6: Perpendicular X-Band EPR spectra of the post-reaction mixture of **2** (black trace) + 1 eq. pTsOH (red trace), + 1.5 eq. pTsOH (green trace), and + 2 eq. pTsOH (blue trace) measured at 2 K (9.65 GHz, 0.2 mW microwave power).*

We determined that 2 eq. of pTsOH resulted in the maximum yield of **4**, however, unreacted **2** was still potentially obscured the complete signal of the new species. $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species, such as **2**, have been found to not display resolved EPR spectra at measurement temperatures of >10 K.^{18,19} Therefore, in order to fully observe the EPR spectra of this new species, EPR spectroscopy of **4** was performed at 30 K (Figure 3.7, blue trace). This resulted in a broadening of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ signal of **2** into the baseline so that the full signal of **4** could be seen. This revealed a 16-line signal centred at $g = 2$ and a 11-line signal at $g = 2.6$. The signal at $g = 2$ was the same as the one observed in the 1 eq. and 1.5 eq. pTsOH EPR spectra, with similar narrow line widths.

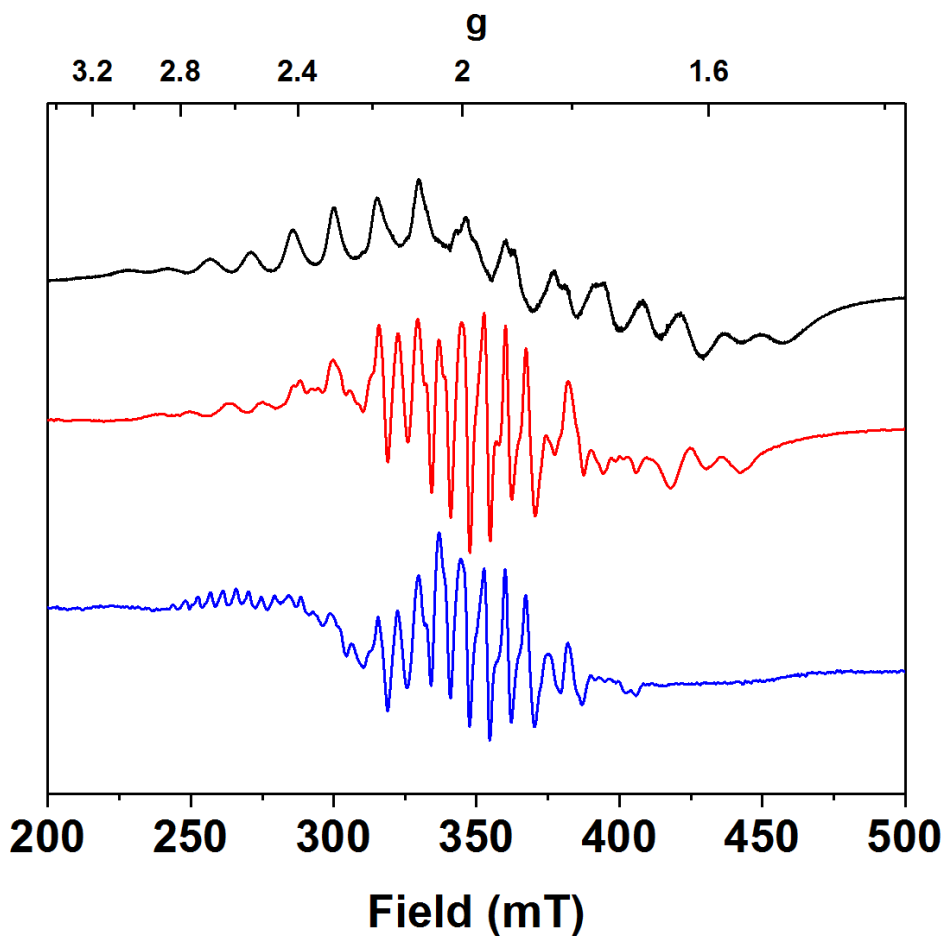


Figure 3.7: Perpendicular X-Band EPR spectrum of the post-reaction mixture of **2** measured at 2 K (black trace), **4** measured at 2 K (red trace) and 30 K (blue trace) (9.65 GHz, 0.2 mW microwave power)

At 30 K, **4** displayed a 16-line signal centred at $g = 2$, characteristic of a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species.²⁰ The number of lines originated from the strong antiferromagnetic coupling between the two Mn centres resulting in a superposition of the lines.²⁰ Interestingly, the line spacing of the **4** was much narrower than that of **2**'s signal with an $A_{\text{av}} = 7.4$ mT for **4** compared to $A_{\text{av}} = 12.4$ mT for **2**. The A_{av} value calculated for **4** was consistent with what was considered characteristic for $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. For example, a $\text{Mn}^{\text{III}}\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{IV}}$ species presented by Pedersen and co-workers reported an A_{av} of 7.8 mT.²¹ The spectral width of the $g = 2$ signal of **4** was only 125 mT, more narrow than the EPR signal of **2** in comparison which spanned over 250 mT. It has been observed

previously in the study of dinuclear Mn complexes and Mn catalase that $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ systems displayed much larger EPR spectral widths in comparison to $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes.²² For example, Dismukes and co-workers also saw a reduction in the EPR spectral width when converting from a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ to a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex.²³ Moreover, the temperature independence of the **4** species at $g = 2$ provided further evidence for the formation of a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ system, as these species have been measured at temperatures up to 77 K.^{24–26} On the other hand, $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ systems required measurement temperatures less than 15 K to be resolved. Therefore, we assigned the $g = 2$ species to be a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex.^{25–27} The yield of the 16-line species was determined by EPR integration to be circa 40 % (A3.13). We postulated that the unreacted $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** in solution contributed to the relatively low-yield of **4**.

EPR studies of **2** + 2 eq. of pTsOH at 30 K also revealed an 11-line feature centred at $g = 2.6$ that was obscured at 2 K by residual $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (Figure 3.7). The 11-line hyperfine signal indicated that the species responsible for this signal contained two Mn atoms that were equivalent $\{(2nI+1) = (2(2)(5/2)+1)) = 11\}$. An A_{av} value of 4.4 mT was determined for this signal which was approximately half of what was determined for the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species **4** (7.8 mT). Interestingly, a similar species, $[\text{Mn}^{\text{II}}_2(\text{TMEDA})_2(\text{OCCH}_3)_4(\text{H}_2\text{O})]$, studied by DeRosa and co-workers displayed an 11-line signal centred at $g = 2.34$ with $A_{av} = 4.5$ mT at 30 K.²⁸ This signal was very temperature sensitive and was not present when the Mn^{II}_2 complex was measured at 6 K. There was a strong resemblance between the signal at $g = 2.6$ and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{II}}(\text{TMEDA})_2(\text{OCCH}_3)_4(\text{H}_2\text{O})]$. Moreover, another Mn^{II}_2 species, $[\text{Mn}^{\text{II}}_2(\text{Me}_3\text{TACN})_2(\mu\text{-OCCH}_3)_3]\text{BPh}_4$, also displayed an 11-line signal at $g = 1.96$ with $A_{av} = 4.6$ mT.²⁹ Therefore, due to 11 hyperfine lines and the A_{av} value with previously studied Mn^{II}_2 systems, we assigned the $g = 2.6$ signal to have originated from a Mn^{II}_2 species.

Interestingly, studies of the Mn^{II}_2 starting material **1**, performed by the Hendrich group, did not display this 11-line Mn^{II}_2 signal we consistently observed at $g = 2.6$.¹² However, variable temperature EPR experiments of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species **2** revealed the 11-line signal when measured > 10 K (A3.12). Therefore, we postulated that upon addition of the oxidant superoxide to **1**, a new Mn^{II}_2 system was formed, possibly through the loss of the acetate bridging ligands and leading to the 11-line signal in the EPR spectrum. The yield of this 11-line signal was found to be negligible via EPR integration, further emphasising that this species was a side-product.

ESI-MS analysis of the just-thawed methanol solution of **4** revealed a peak at $m/z = 335.5837$ consistent with the calculated peak of $m/z = 335.5682$ for the dication $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}\text{bis}(\mu\text{-oxo})(\text{BPMP})]^{2+}$ (Figure 3.8, left). This peak was not observed in the mass spectrum of the precursor **2** (A3.15). Preparation of **2** using K^{18}O_2 followed by activation with 2 eq. of pTsOH to yield a potentially isotopically labelled form of complex **4**, resulted in the mass peak to shift by two mass units ($m/z = 337.5788$), consistent with the incorporation of two ^{18}O atoms into the dicationic ion (Figure 3.8, right). This observation suggested that complex **4** contained two O atoms derived from KO_2 and indicated the presence of oxo-core in the molecule.

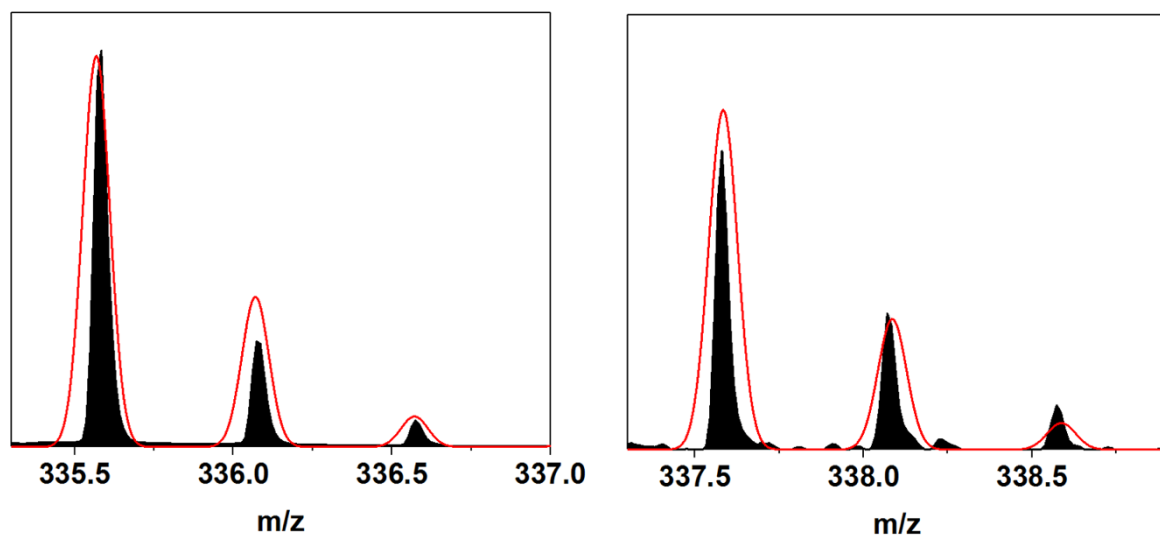


Figure 3.8: Left; ESI-MS spectra of **4** prepared using $K^{16}O_2$ (black trace) with simulated spectra (red trace). Right; ESI-MS spectra of **4** prepared using $K^{18}O_2$ (black trace) with simulated isotopic spectra (red trace).

Mn K-edge X-ray absorption near edge spectroscopy (XANES) was performed on **2** and **4** in order to gain an understanding of the oxidation states of the metal centres and their electronic symmetry and local geometry (Figure 3.9). The XANES spectra of the $Mn^{II}Mn^{III}$ -peroxide **2** displayed a rising edge with first inflection point calculated to fall at 6548.9 eV (Figure 3.9, black trace). **2** also displayed a pre-edge transition that was modelled using two 50:50 pseudo-Voigt functions and determined to be centred at 6540.6 eV with an area of 13.5 units (A2.4). The first inflection point of new species **4** was calculated to fall at 6549.4 eV (Figure 3.9, red trace). **4** also displayed a pre-edge transition that was modelled using two 50:50 pseudo-Voigt functions that determined the peak was centred at 6540.5 eV with an area of 10.2 units (A3.17).

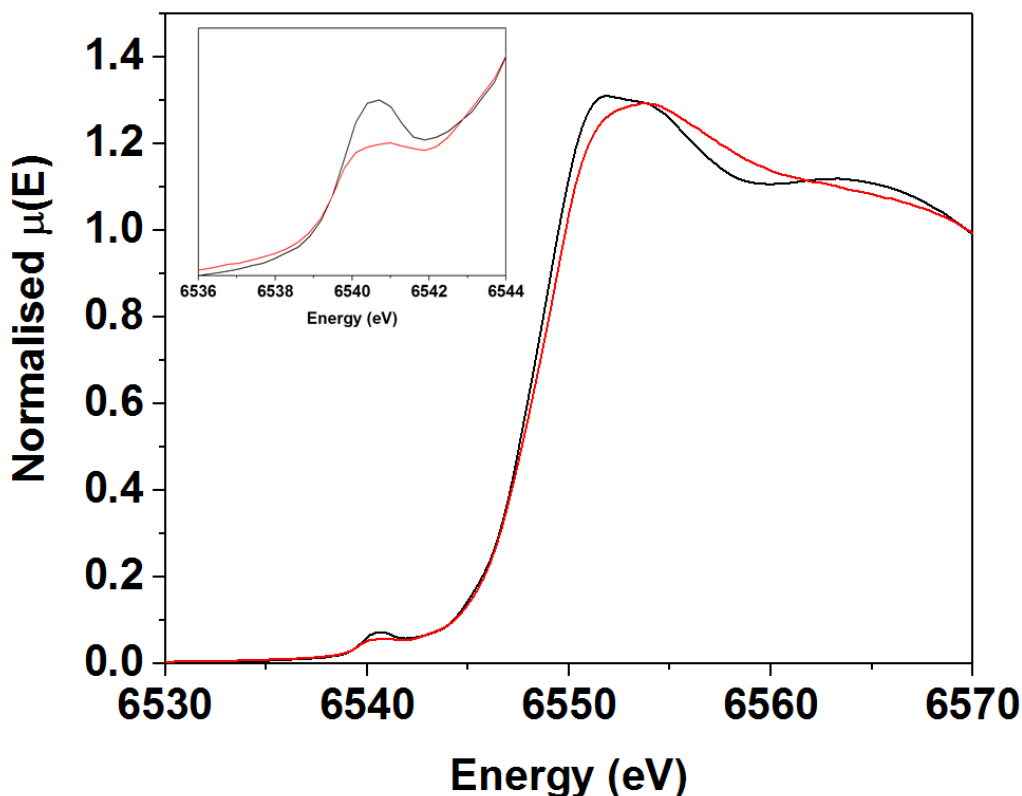


Figure 3.9: Normalised XANES spectra of **2** (black trace) and **4** (red trace). Inset: Expansion of the pre-edge region.

Interestingly, with the conversion of **2** to **4** a decrease in the intensity of the shoulder at ~6552 eV was observed. This was indicative of the decrease in concentration of Mn^{II}_2 present in the sample, and has been observed previously in XANES studies of Id RNRs.³⁰ This demonstrated that less Mn^{II}_2 was present in **4** compared to **2**, and indicated the conversion to higher oxidation states in **4**. Upon conversion from **2** to **4** the edge energy shifted from 6548.9 eV to 6549.4 eV, a change of just 0.5 eV. An increase in the oxidation number of Mn by one was typically associated with an edge energy shift of 2 - 4 eV.^{31,32} As XANES is a measure of the average of the oxidation states of all the Mn centres in the sample we postulated the unreacted $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide of **2**, as observed in the EPR experiments, affected the edge energy of sample **4**.¹⁷ The low yield of **4** determined by EPR analysis also explained the relatively low edge energy shift observed in the XANES spectra. Although the value for edge energy shifted just by 0.5 eV, the edge energy value determined

for **4** was consistent with that determined for a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ in the enzyme Mn catalase of 6549.2 eV.³³

The peak before the main edge, the pre-edge, was associated with crystal field transitions from the core 1s to 3d orbitals. Where this peak falls and the area of pre-edge can give valuable information about the local structure of the Mn centres. The pre-edge areas of **2** and **4** were determined to be 13.5 and 10.2 units respectively (Table 3.1). A decrease in the pre-edge area was associated with an increase in electronic symmetry of metal centres.³¹ A possible explanation for the decrease in area observed upon conversion from **2** to **4** was that the Mn atoms in **2** were suggested to be pentacoordinate by mass spectrometry studies (Chapter 2), while if **4** was a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ system, as EPR investigations suggested, the metal centres adopt a hexacoordinate geometry. Hexacoordinated Mn centres are inherently more centrosymmetric than pentacoordinate ones, and therefore have more electronic symmetry. Therefore, the decreased pre-edge area of **4** relative to **2**, was indicative of the formation of a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex. Interestingly, the pre-edge area of **4** was still larger than that of **1**, even though the Mn centres in both complexes are postulated to have six coordinating ligands (Table 3.1). This was rationalised as the higher oxidations of a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ system in **4** would lead to more distortion around the metal centres compared to the lower valent **1**, leading to a slight increase in pre-edge features even though both complexes had similar coordination numbers.³¹

Table 3.1: XANES Data for Complexes **1**, **2** and **4**.

Complex	Edge Energy (eV)	Pre-Edge Area (units)	Pre-Edge Peak (eV)
1	6548.4 ($\pm$ 0.1)	8.8 ($\pm$ 0.1)	6540.4 ($\pm$ 0.1)
2	6548.9 ($\pm$ 0.1)	13.5 ($\pm$ 0.1)	6540.6 ($\pm$ 0.1)
4	6549.4 ($\pm$ 0.1)	10.2 ($\pm$ 0.1)	6540.5 ($\pm$ 0.1)

The bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex **4** was formed following the reaction of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** with the acid pTsOH, therefore, we were interested in understanding if the bis(μ -oxo) core of **4** contained a proton. To investigate this, **4** was reacted with the base triethylamine (NEt_3) and the reaction was monitored using UV-vis spectroscopy. Upon addition of NEt_3 to **4** an immediate blue-shift of the bands associated with **4** was observed (Figure 3.10). Pecoraro *et al.* have performed studies on how the electronic absorption features of bis(μ -oxo) Mn^{IV}_2 species change as the core became protonated.³⁴ Pecoraro observed that moving from a bis(μ -hydroxo) to (μ -oxo)(μ -hydroxo) to bis(μ -oxo) species, the main feature in the UV-vis spectrum, attributed to phenolate-to-Mn charge transfer, shifted towards shorter wavelengths. The (μ -oxo)(μ -hydroxo) Mn^{IV}_2 species displayed this intense feature at $\lambda_{\text{max}} = 550$ nm, the same λ_{max} as the main band in the spectrum of **4** that became blue-shifted upon conversion to the bis(μ -oxo) Mn^{IV}_2 species. Therefore, in analogy with the work done by Pecoraro, we postulated that the feature at $\lambda_{\text{max}} = 550$ nm in the UV-vis spectrum of **4** was the result of phenolate-to-Mn charge transfer from a (μ -oxo)(μ -hydroxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (**4**) and converted to a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species following reaction with a base.

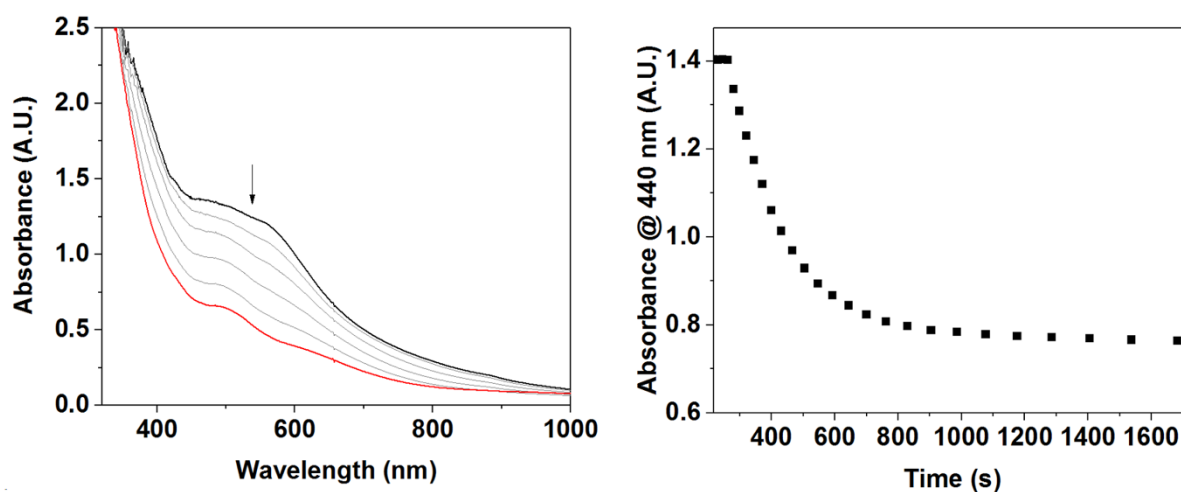


Figure 3.10: Left: UV-vis absorption spectra of the reaction of **4** + 50 eq. NEt_3 in 1:9 MeCN at -90°C . Right: Plot of the absorbance at 440 nm versus time during the reaction of **4** and NEt_3 .

With the combination of UV-vis, EPR, X-ray absorption spectroscopies, mass spectrometry, and base studies new species **4** was assigned to be a $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. We postulated that addition of the weak acid pTsOH as a proton source facilitated O-O bond scission of the peroxo species **2** and promoted conversion to the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (Figure 3.11). In fact, in close analogy to the conversion of **2** to **4**, Que and co-workers recently reported the cleavage of a $(\mu\text{-1,2-peroxo})\text{Fe}^{\text{III}}_2$ species upon addition of the Lewis acid Sc^{3+} to generate a bis($\mu\text{-oxo})\text{Fe}^{\text{IV}}_2\text{core}$.³⁵ Que postulated that upon reaction with Sc^{3+} , the peroxide isomerised from the $\mu\text{-1,2}$ binding to a $\mu\text{-}\eta^2\text{:}\eta^2$ coordination mode, which then allowed for O-O cleavage to form to generate the bis($\mu\text{-oxo})\text{Fe}^{\text{IV}}_2$ species.

The different UV-vis and EPR spectra produced when **2** was reacted with a strong acid (HCl) to form **3** versus a weak acid (pTsOH) to form **4**, indicated that a different mechanism was employed depending on the strength of the acid. This agreed with the case of a Mn^{IV} -peroxo complex reported by Nam and co-workers, in which it was proposed that the Mn-O or the O-O bond was cleaved depending on whether a strong to weak acid was used.³⁶

We postulated that a similar mechanism was also occurring with **2**, whereby, in the presence of strong acids a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{-OOH}$ species was formed that subsequently undergoes Mn-O bond cleavage resulting in a mixed-valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ and H_2O_2 (Figure 3.11). In contrast, when **2** reacted with a weak acid a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{-OOH}$ species was formed that underwent intermolecular O-O bond cleavage and a $(\mu\text{-hydroxo})(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ entity was produced.

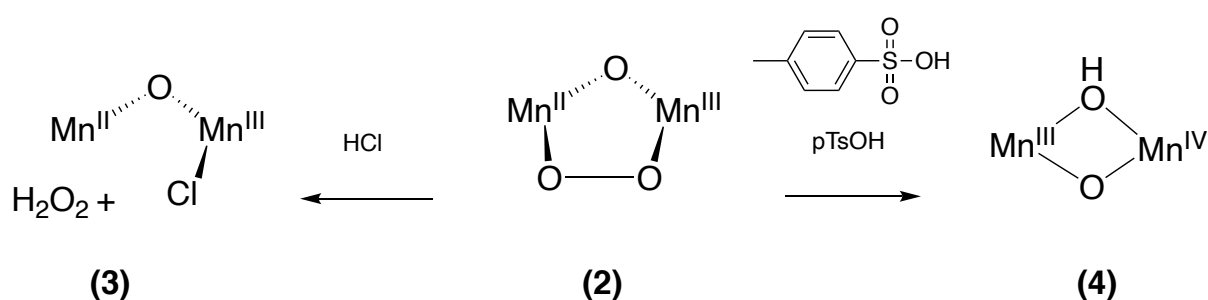


Figure 3.11: Postulated adducts formed upon reaction of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**) with HCl (forming **3**) and pTsOH (forming **4**).

3.4 Reactivity of Species 4

In the catalytic cycle of Ib RNRs, it was postulated that a $(\mu\text{-hydroxo})(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species was responsible for the oxidation of a nearby tyrosine residue to a tyrosyl radical thereby initiating ribonucleotide reduction.⁵ Complex **4** had a similar structure to this postulated intermediate in Ib RNRs, therefore, we were interested in investigating if **4** was capable of replicating similar O-H bond reactivity observed in the enzyme. The *para* substituted phenol 4-methoxy-2,6-di-*tert*-butylphenol (4-OCH₃-2,6-DTBP) was chosen as a functional mimic of the tyrosine residue. Addition of 50 eq. of 4-OCH₃-2,6-DTBP to **4** at -90 °C resulted in the decay of the electronic absorption features associated with the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species **4** and the simultaneous growth of a sharp feature at $\lambda = 405$ nm (Figure 3.12, left). This new sharp feature has been observed before in the reaction of metal complexes and 4-OCH₃-2,6-DTBP and was attributed to the 4-methoxy-2,6-di-*tert*-

butylphenoxy radical species.³⁷ The 4-methoxy-2,6-di-*tert*-butylphenoxy radical was independently synthesised (A3.18) and a UV-vis spectrum of the radical was measured in the same solvent system and temperature as the reaction between **4** and the phenol (A3.19). The radical species displayed a UV-vis feature at $\lambda_{max} = 405$ nm with the same shape as the feature formed during the reaction of **4** and the phenol. Unfortunately, the radical was found to be unstable in the solvent system and began to decay immediately once dissolved, meaning that a yield for the radical species could not be determined via UV-vis spectroscopy. EPR measurement of the post-reaction mixture of **4** and 4-OCH₃-2,6-DTBP revealed an intense, sharp, isotropic signal centred at $g = 2$, indicative of the formation of an organic radical species (Figure 3.12, right). Therefore, it was determined that the 4-methoxy-2,6-di-*tert*-butylphenoxy radical was the product of the reaction. A yield of 35% $\pm$ 15% was determined for the radical considering a one-electron oxidation using EPR spectroscopy. Interestingly, no metal-based signals were observed in the post-reaction EPR recorded at 77 K. Although the organic based phenoxy radical signal is quite intense, it was also a very narrow signal compared to those observed for dinuclear Mn species. Despite this, no metal based signals could be discerned from the spectrum. This implied the formation of an EPR silent Mn₂ product following reaction with 4-OCH₃-2,6-DTBP. Analogously, in the catalytic cycle of Ib RNRs, the EPR spectrum recorded following reaction with tyrosine also displayed no metal based signals and only the signal of the tyrosyl based organic radical was visible.³⁸ Subsequent analysis of Ib RNRs revealed the formation of Mn^{III}₂ complex, which was EPR silent as it had an integer value of S_T . Therefore, we proposed that following the reaction with 4-OCH₃-2,6-DTBP, **4** converted to an EPR silent Mn^{III}₂ species.

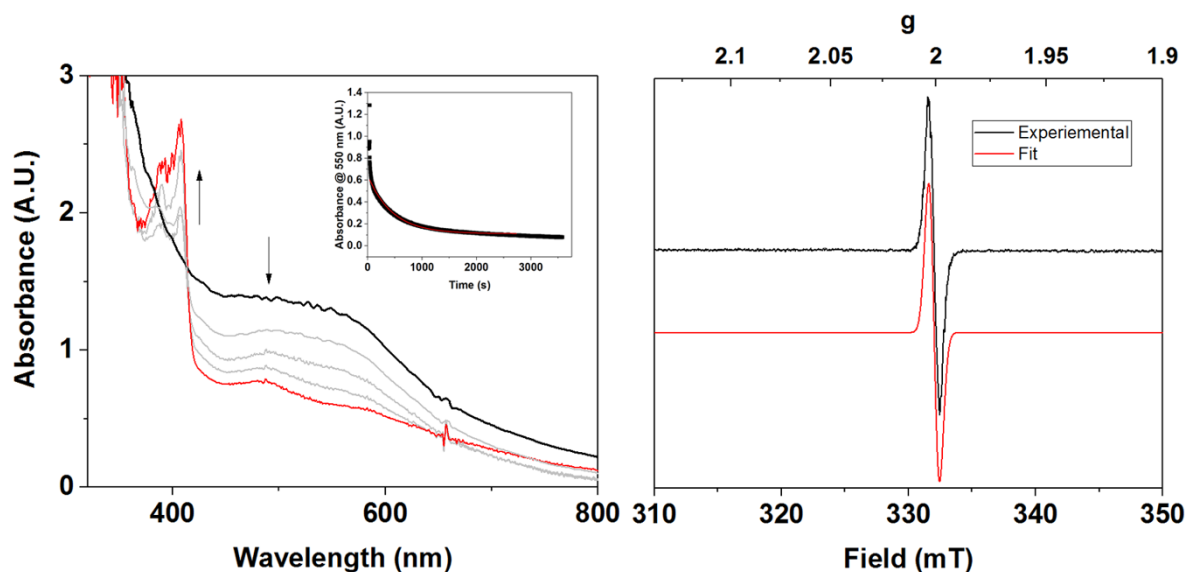


Figure 3.12: Left: electronic absorption spectra of the reaction of **4** (black) with 150 eq. 4-OCH₃-2,6-DTBP (red). Inset: Plot of the change of absorption at 550nm over time fitted as a pseudo first-order reaction. Right: Perpendicular X-Band EPR spectrum of the reaction of **4** and 4-OCH₃-2,6-DTBP measured at 77 K (9.65 GHz, 0.2 mW microwave power) in black and the simulated spectra in red.

In order to gain mechanistic insight, **4** was reacted with other O-H bond containing substrates such as 9-azabicyclo[3.3.1]nonane-N-hydroxide (ABNO-H) and substrates from the 1-hydroxy-2,2,6,6-tetramethyl-piperidines (TEMPO-H) family. Addition of these substrates to **4** resulted in the decay of the electronic absorption features associated with **4**. Frozen samples of the post-reaction mixtures were analysed via EPR spectroscopy and each sample displayed an intense rhombic signal characteristic of the formation of the corresponding ABNO $\cdot$ and TEMPO $\cdot$ radicals (A3.22, A3.25, A3.29, A3.31, and A3.34). The yields of the radical species were determined using EPR spectroscopy, assuming one-electron oxidation (Table 3.2). In the ESI-MS spectrum of the post-reaction mixture of **4** and TEMPO-H the peak associated with **4** ($m/z = 335.5837$) was no longer present (A3.36). This provided further evidence for **4** being the active species responsible for oxidation of the O-H bonds, as **4** would be consumed during the reaction with TEMPO-H, thereby no peak associated with the complex in the mass spectrum was observed.

Table 3.2: Kinetic parameters for the radical forming reaction of complex **3** with O-H bond containing substrates.

Substrate	k_2 ($M^{-1} s^{-1}$)	EPR Radical Yield ($\pm 15\%$)	BDFE ($kcal\ mol^{-1}$)^a
4-OCH ₃ -TEMPO-H	0.258 (± 0.05)	60%	65
TEMPO-H	0.150 (± 0.003)	75%	66.5
4-oxo-TEMPO-H	0.134 (± 0.007)	75%	65.6
ABNO-H	0.018 (± 0.001)	40%	71.5 ^b
[H]-4-OCH ₃ -2,6-DTBP	0.0072 (± 0.0002)	35%	74.9
[D]-4-OCH ₃ -2,6-DTBP	0.0028 (± 0.0001)	-	-

^aThe BDFE values of each substrate are reported in MeCN.^{39–41} ^bBDFE values for ABNO-H were not available in MeCN, and hence the value was derived using gas phase bond dissociation energies. Details in Appendix.

The kinetic investigation of **4** was performed under *pseudo* first-order conditions. Fitting of the decay of the complex by monitoring a UV-vis feature at $\lambda_{max} = 550$ nm associated with **4** after addition of > 10 eq. of substrate afforded a *pseudo* first-order rate constant (k_{obs}) (Figure 3.12, left, inset). A plot of the k_{obs} versus substrate concentration revealed a linear relationship and fit to obtain a second-order rate constant (k_2) from the slope (A3.21). Determining a k_2 value for **4** for a specific substrate such as TEMPO-H allowed for

comparison with similar Mn complexes (Table 3.3). **4** was found to be slower than the similar $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex synthesised by Kovacs, however, this complex was measured at a temperature 17 °C higher than **4**.⁴² This could be the source of the difference of rate measurements between the two complexes. Unfortunately, to the best of our knowledge, the k_2 values of the reaction between TEMPO-H and other $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes have not been reported, limiting our comparison. The reported k_2 values for Mn^{III} hydroxide complexes were 2-3 orders of magnitude higher than that of **4** (Table 3.3). However, again the Mn^{III} -hydroxides were measured at much higher temperatures than **4**, a difference of 110 °C, which explained the differences in reaction rate. No reported k_2 values were found for the reaction of Mn^{IV} -oxo species with TEMPO-H, likely because the reaction proceeded quite quickly. Jackson and co-workers reported in their studies that the k_2 values of Mn^{IV} -oxo units were more reactive than $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species with a common phenol substrate by a factor of 10^5 .⁴³ From this we inferred that a Mn^{IV} -oxo unit would react much faster than **4**. One argument as to why nature employed a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ dinuclear species in Ib RNRs even though it appeared to be less reactive than a mononuclear counterpart, would be to minimise enzyme damage during the tyrosine oxidation step.⁴⁴ High valent mononuclear Mn-oxo species can exist long enough to do irreversible oxidative damage to the protein active site rendering the enzyme ineffective.⁴⁵ Alternatively, the Mn-oxo species could react with the ribonucleotide substrates damaging the products of the enzyme. Enzymes must therefore choose an active oxidant that fits in a “Goldilocks” zone: reactive enough to complete the required biochemistry, but not so reactive as to damage the biological machinery. This phenomenon was observed during studies of the cytochrome (Cyp) P450 enzymes which contained a cysteine ligated heme in the active site and were capable of catalysing oxidative reactions of wide variety of endogenous and exogenous substrates.⁴⁶ The active oxidant in the catalytic cycle of Cyp P450 is an intermediate termed compound I that contained a Fe^{IV}

oxo species. The higher pK_a of the oxo ligand in compound I resulted in a decrease in the redox potential of the species thus limiting oxidative damage.⁴⁵ Without this tuning effect of the oxo ligands, the Fe^{IV} -oxo centre would certainly have reacted with the amino acid side chains within the active site, destroying the proteins function. Therefore, the reactivity of Cyp P450 although powerful, was intentionally tuned lower by nature to prevent deleterious reactions. We postulated that a similar phenomenon occurred in the enzyme Ib RNRs, whereby a dinuclear manganese active species was utilised over a mononuclear one in order to minimise protein damage during the catalytic cycle.

Table 3.3: Rate constants for the oxidation of TEMPO-H by various Mn complexes

Complex	TEMPO-H k_2 ($M^{-1} s^{-1}$)	Temperature ($^{\circ}C$)	Reference
4	0.150 ($\pm$ 0.003)	-90	This work
$Mn^{III}(\mu-O)(\mu-OH)Mn^{IV}(MeOpy)$	0.938	-73	42
$[Mn^{III}(OH)(dpaq^{2Me})]^+$	31	25	47
$[Mn^{III}(S^{Me}N_4(tren))(OH^+)]^+$	210	25	48

In order to determine if the initial oxidation of the substrate occurred via a stepwise or concerted PCET mechanism the free energy of activation of the reactions ($\Delta G^\ddagger$) were calculated using the Eyring equation and using these values a Bell-Evans-Polyani (BEP) plot was constructed (Figure 3.13). A linear fit of the BEP plot afforded a slope of 0.16. This was relatively close to the value of 0.5 predicted by Marcus theory for hydrogen atom transfer (HAT) and falls within the range of $\Delta G^\ddagger$ vs $BDFE_{O-H}$ slopes (0.15 - 0.7) associated with HAT mechanisms.^{41,49} Furthermore, a stepwise reaction PCET reaction with TEMPO-

H was thermodynamically unlikely as there was a high barrier to initial deprotonation or one-electron oxidation ($pK_a = 41$ and $E^\circ = 0.71$ V vs $\text{FeCp}_2^+/\text{FeCp}_2$ in MeCN).⁴¹ Therefore, oxidation of TEMPO-H must have proceeded via a HAT mechanism unless it reacted with an exceptionally strong base or one-electron oxidant.⁵⁰

A kinetic isotope effect (KIE, k_H/k_D) of 2.5 was determined for the reaction of **4** with O-H bonds using *OD*-4-OCH₃-2,6-DTBP (A3.43). This KIE value falls within the classical range (2-7) and indicated that proton or hydrogen atom transfer was involved in the rate determining step. This value of KIE determined for **4** was in agreement with the KIE of 3.5 observed for the similar complex $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{MeOpy})$ reported by Kovacs and co-workers, that was also found to undergo HAT.⁴² The similarities between $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{MeOpy})$ and **4** offered further indication **4** utilised a HAT mechanism during oxidation of O-H bond containing substrates.

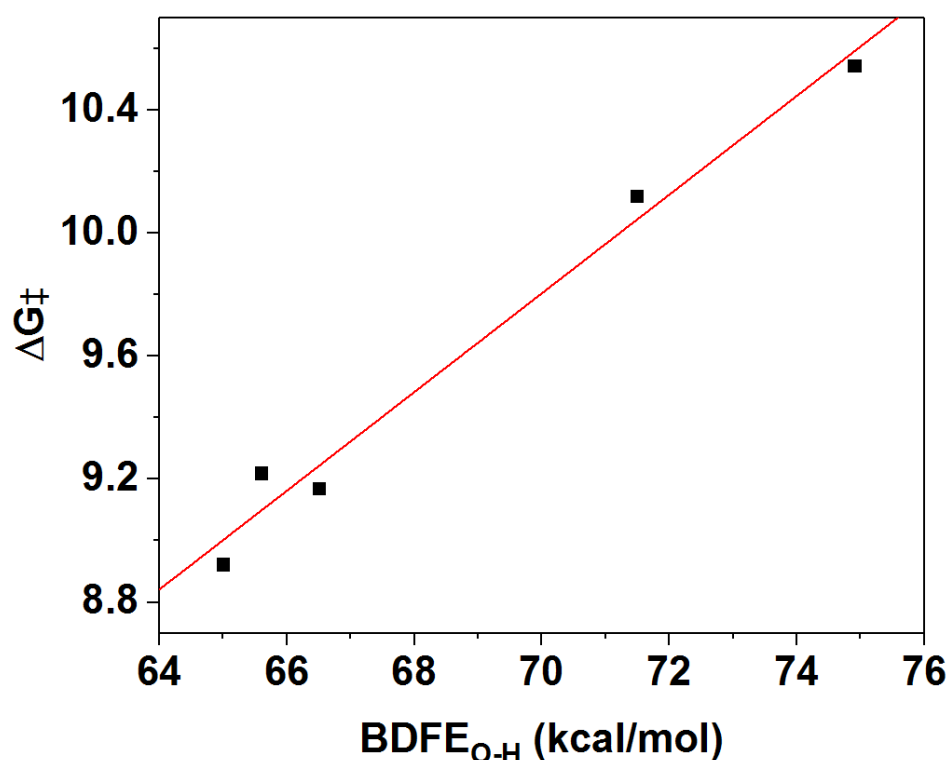


Figure 3.13: BEP plot for the reaction of **4** with O-H bond containing substrates (slope = 0.16). Values of $\Delta G^\ddagger$ were calculated from first-order constants k_1 via the Eyring equation. BDFE values of the substrates are provided are plotted in MeCN.

3.5 The Mechanistic Implications for Ib RNRs

The structure and reactivity of **4** had a number of implications for the mechanism of class Ib RNRs that may provide some insight to the activity of the enzyme in vivo. Firstly, **4**'s arrangement as a diamond core $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ provided additional evidence for the identity of the active species in the catalytic cycle to also assume a similar structure. To date, only a 16-line frozen EPR signal spectra was provided in support of a $(\mu\text{-hydroxo})(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ active species.⁵ We have now provided further experimental evidence in support of a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ oxidant.

Furthermore, **4** was generated from a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**) upon activation with a proton. This was especially interesting for the catalytic cycle of Ib RNRs as many questions linger around the identity of the species preceding generation of the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ active oxidant. This unknown intermediate has never been trapped in the native enzyme and no spectroscopic evidence exists of the structure.⁵ Previously, our group has postulated that a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide would be an ideal candidate for this intermediary as we have generated two different mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ peroxides from Mn^{II}_2 complexes upon reaction with superoxide, the activating oxidant in the catalytic cycle of Ib RNRs.^{10,11} Moreover, this work explained that the role of the postulated proton in the cycle may be to activate the conversion of an $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide to the active oxidant $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species, similar to how we observed formation of **4** upon addition of pTsOH to **2**. Finally, the kinetic studies of the reactivity of **4** demonstrated that the dimanganese cofactor in the native enzyme may have utilised a HAT mechanism to generate the tyrosyl radical species responsible for initiating ribonucleotide reduction. It has long been postulated that RNRs would undergo a concerted PCET to synthesise the tyrosyl radical species to circumvent intermediary high-energy charged species that could damage the protein structure.⁷ However, to the best of

our knowledge, no experimental evidence for Ib RNRs enzymes employing this mechanism has been provided. Our model complex **4** provided the first case of empirical evidence for concerted HAT to occur during generation of the tyrosyl radical.

3.6 Conclusions

The complex $[(\text{Mn}^{\text{II}})_2(\text{BPMP})(\text{OAc})_2(\text{ClO}_4)]$, (**1**), was reacted with superoxide to form a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**). **2** reacted with the strong acid HCl to generate the new species **3** observed via UV-vis spectroscopy. The electronic absorption features of **3** were comparable to previously studied $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes. EPR investigations into **3** proved unsuccessful due to saturation of the signal from free Mn^{II} ions.

2 was also found to react with the weak acid species pTsOH to form a new $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (**4**). The structure of **4** was verified by EPR, UV-vis, and X-ray absorption spectroscopies and mass spectrometry studies. **4** displayed similar EPR spectra to the implicated active species identified in studies of Ib RNRs. **4** was also found capable of replicating the reactivity of the enzyme by oxidising O-H bonds to the oxygen radical species. Kinetic analysis revealed that **4** was reacting with the substrates via a HAT mechanism whereby the proton and the electron were transferred in a single, concerted step. This not only provided further support for a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species as the active oxidising species in the catalytic cycle in Ib RNRs but also provided the first experimental support for preference of a HAT mechanistic oxidation of the tyrosine residue in the enzyme.

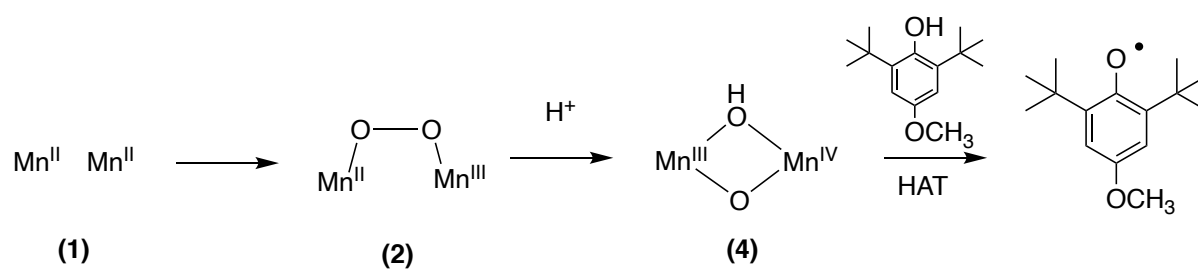


Figure 3.14: Graphical Conclusion

3.7 Experimental

Materials

All reactions with air-sensitive materials were performed in a glove box in a N₂ atmosphere or under inert conditions using inert Schlenk line techniques. All reagents and solvents were purchased from commercial sources. Anhydrous *N,N*-dimethylformamide (DMF) was purchased and used without further purification. Anhydrous tetrahydrofuran (THF), diethyl ether (Et₂O), and acetonitrile (MeCN) were dispensed through an MBraun MB-SPS-5 Solvent Purification System and deoxygenated by purging with argon.

N,N-bis(2-picoyl)amine and 2,6-bis(chloromethyl)-4-methylphenol were synthesised as previously described.^{51,52} The ligand 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol (HBPMP) was synthesised as previously reported.⁵³ The synthesis of [Mn₂(O₂CCH₃)₂(BPMP)](ClO₄), **1**, was carried out as previously reported.⁵⁴

Caution: KO₂ and perchlorate salts of metal complexes are potentially explosive and must be handled with care and in small quantities. No problems were encountered during this project.

Physical Methods

¹H NMR analysis was performed on a Bruker Avance III 400 MHz instrument. Electrospray ionisation mass spectra (ESI-MS) were obtained using micromass time of flight spectrometer (TOF), interfaced to a Waters 2690 HPLC, or by direct injection in the mass spectrometry instrument. Electronic absorption spectra were recorded in quartz cuvettes on a Hewlett Packard (Agilent) 8453 diode array spectrophotometer (190 – 1100 nm range) coupled to a liquid nitrogen cooled cryostat from Unisoku Scientific Instruments (Osaka, Japan). Electronic paramagnetic resonance (EPR) measurements were conducted on a Bruker Eleksys E-500 spectrometer with an Oxford ESR 910 liquid helium cryostat

and an Oxford temperature controller. The 30 K EPR quantification of the signals was relative to a $[\text{Cu}(\text{NO}_3)_2]$ spin standard in CH_3OH . The 77 K EPR quantification of the signals were relative to a TEMPO $\cdot$ spin standard in 1:9 MeCN/THF. EPR spectra of 4-X-TEMPO and ABNO were recorded at 77 K, 9.2 GHz, 2.02 mW microwave power, with a 60 mT field sweep in 84 s, and 0.3 mT field modulation amplitude. Integration, simulation, and fitting were performed with Matlab and the easySpin computational package.⁵⁵ The simulation for the phenoxyl radical spectra was modelled as an $S = \frac{1}{2}$ electron spin with an isotropic g tensor.

X-ray absorption spectroscopy methods: The Mn-K-edge X-ray absorption data were collected on beamline 7-3 of the Stanford Synchrotron Radiation Lightsource (SLAC National Accelerator Lab, Menlo Park, CA, USA). Data were collected with the storage ring operating at 3.0 GeV and 500 mA, using a LN2 cooled Si(220), $\phi = 90^\circ$ double-crystal monochromator, calibrated by using the first inflection point of a Mn foil (6539.0 eV). The monochromator was detuned by $\sim 50\%$ for higher harmonic rejection. A Canberra 32-element solid state germanium detector was used for fluorescence detection. All the measurements were performed at ambient pressure at ~ 17 K using an Oxford helium cryostat that was cooled by closed-cycle He gas loop. The parameters used for the scans were the following: 10 eV steps/1 second integration time in the pre-edge region, 0.3 eV steps /2 second integration time in the edge, and 0.05k steps in the EXAFS, with integration time increasing in a k^2 -weighted fashion from 2 to 4 seconds over the energy range ($k_{\text{max}} = 12.1k$). The total detector counts were typically 3-7k, well within the linear range of the detector electronics. Each sample was monitored for photoreduction. Complex **2** and **4** were found to be photoreduced by the X-rays and so only one scan was obtained per spot. Delrin[®] Mossbauer/XAS cups with sample windows of 4 mm x 10 mm.. Elaboration of the XAS data, including averaging, background removal and normalization, was performed using

Athena.⁵⁶ Edge energies were obtained by taking the first derivative of the rising edge, while pre-edge energies were identified using the second derivative. Peak fitting analysis employed the program Fityk,⁵⁷ using the protocol developed by Westre et al. for analysing Fe K-edge pre-edge peaks.⁵⁸ Briefly, a 15-18 eV segment of the normalized $\mu(E)$ spectrum was fit around the pre-edge, with pre-edge peaks modelled using pseudo-Voigt functions having a fixed 50:50 mixture of Gaussian: Lorentzian character, while the background absorption was modelled with a single pseudo-Voigt function where the proportion of Gaussian: Lorentzian character was allowed to float. Four slightly different energy ranges were fit separately, and the results averaged to generate the reported peak fits.

Experimental Procedures

General procedure for the reaction of 1 with KO₂ and cryptand

A 1:9 CH₃CN/THF solution (1.5 mM) of complex **1** was prepared. In a quartz cuvette 2 mL of a 1.5 mM solution of complex **1** was cooled to -90 °C. KO₂ (0.0056 g, 0.04 M) and cryptand (0.06 g, 0.08 M) were dissolved in DMF (2 mL). 75 μ L of this solution was added to the solution containing **1**. The reaction progress was monitored using electronic absorption spectroscopy. Lower yields were observed (less intense absorption features) with high equivalencies of KO₂ and at higher concentrations of **1**.

General Procedure for the reaction of 2 with pTsOH

A solution of **2** was prepared in a quartz cuvette at -90 °C as outlined in the procedure above. pTsOH (0.095g, 0.55 M) was dissolved in 1:9 MeCN/THF. 12 μ L of this solution was added to the solution containing **1**. The reaction progress was monitored using electronic absorption spectroscopy.

Synthesis of ABNO-H

ABNO-H was synthesised using an adapted procedure from the literature.⁵⁹ Na₂S₂O₄ (1.00g, 5.7 mmol) was added to a dry, degassed solution of ABNO (500 mg, 3.7 mmol) dissolved in 15 ml of 1:1 acetone/water solution. Solution turned from orange to white. Acetone was removed under vacuum and the solution was extracted with dry, degassed pentane (3 x 10 ml). The white precipitate was dissolved in ~15 ml diethyl ether and solvent removed under vacuum (342 mg, 70% yield). The ¹H NMR spectrum was consistent with previously published spectra of ABNO-H.⁶⁰

EPR Sample Preparation

Samples for EPR analysis were prepared by transferring ~ 1 mL of the desired solution from the quartz cuvette at -90 °C, via a pre-cooled pipette into a pre-cooled EPR tube and immediately freezing it in liquid nitrogen. The EPR spectra were recorded at 2 K (9.64 GHz, 0.2 mW microwave power).

Frozen Mass Spectrometry Sample Preparation

The samples for frozen MS analysis were prepared by transferring ~ 0.1 mL of the desired solution from the quartz cuvette, pre-cooled to -90 °C, via a pre-cooled MS sample vial with pre-cooled methanol and immediately freezing in liquid nitrogen. ESI- MS was performed on the just cooled sample.

Reactivity Studies

A solution of **2** in 1:9 CH₃CN/THF at -90 °C was prepared using the above procedure with Cryptand. Substrates were added as concentrated CH₃CN/THF solutions to solutions of **2** at -90 °C. The reactions were monitored using electronic absorption spectroscopy.

Preparation of $K^{18}O_2$:

Method was adapted from a similar procedure reported by Tolman *et al.*⁶¹ TEMPO-H (0.159 g, 1 mmol) was reacted with potassium hydride (KH) in 2 ml of THF. Solution was allowed to stir for two hours until gas evolution ceased. Solution was filtered through a 0.2 μ m syringe filter and the filter washed with an addition 2 ml THF. Solution freeze pump thawed to ensure an environment free of $^{16}O_2$. The solution was frozen in liquid nitrogen and the headspace evacuated and refilled with $^{18}O_2$. Solution was allowed to warm to room temperature and upon this changed from a colourless solution to a dark orange colour. The solvent was removed under vacuum resulting in a dark orange residue. The residue was washed with dry, degassed hexane to yield a yellow precipitate. The hexane was decanted and the powder was washed several times with hexane until the decanted liquid was completely colourless. The yellow powder was then dried under vacuum and used without further purification.

Synthesis of 4-methoxy-2,6-di-tert-butylphenoxy radical

4-methoxy-2,6-di-tert-butylphenoxy radical was synthesised according to a previously reported procedure.⁶² The purple oil obtained was washed three times with deoxygenated diethyl ether (Et_2O), the solvent removed and the oil dried under vacuum for one hour. It was then stored in a nitrogen atmosphere glovebox where the solutions for electronic absorption spectroscopy were prepared in deoxygenated 1:9 MeCN/THF.

3.8 References

- (1) Eriksson, M.; Jordan, A.; Eklund, H. *Biochemistry* **1998**, 37 (38), 13359–13369.
- (2) Aye, Y.; Li, M.; Long, M. J. C.; Weiss, R. S. *Oncogene*. Nature Publishing Group June 9, 2014, pp 2011–2021.
- (3) Cotruvo, J. A.; Stubbe, J. *Annu. Rev. Biochem.* **2011**, 80 (1), 733–767.

- (4) Kolberg, M.; Strand, K. R.; Graff, P.; Kristoffer Andersson, K. *Biochim. Biophys. Acta - Proteins Proteomics* **2004**, 1699 (1–2), 1–34.
- (5) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, 135 (10), 4027–4039.
- (6) Barman, P.; Upadhyay, P.; Faponle, A. S.; Kumar, J.; Nag, S. S.; Kumar, D.; Sastri, C. V.; de Visser, S. P. *Angew. Chemie - Int. Ed.* **2016**, 55 (37), 11091–11095.
- (7) Minnihan, E. C.; Nocera, D. G.; Stubbe, J. A. *Acc. Chem. Res.* **2013**, 46 (11), 2524–2535.
- (8) Kang, G.; Taguchi, A. T.; Stubbe, J. A.; Drennan, C. L. *Science*, 2020, 368(6489), 424–427
- (9) Zhang, F.; Cady, C. W.; Brudvig, G. W.; Hou, H. J. M. *Inorganica Chim. Acta* **2011**, 366 (1), 128–133.
- (10) Magherusan, A. M.; Kal, S.; Nelis, D. N.; Doyle, L. M.; Farquhar, E. R.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2019**, 58 (17), 5718–5722.
- (11) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, 57 (4), 918–922.
- (12) Diril, H.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S.; Chang, H. R.; Nilges, M. J.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, 111 (14), 5102–5114.
- (13) Chaignon, J.; Stiriba, S. E.; Lloret, F.; Yuste, C.; Pilet, G.; Bonneviot, L.; Albela, B.; Castro, I. *Dalton. Trans.* **2014**, 43 (25), 9704–9713.
- (14) Boelrijk, A. E. M.; Khangulov, S. V.; Dismukes, G. C. *Inorg. Chem.* **2000**, 39 (14), 3009–3019.
- (15) Banu, K. S.; Chattopadhyay, T.; Banerjee, A.; Mukherjee, M.; Bhattacharya, S.; Patra, G. K.; Zangrando, E.; Das, D. *Dalton. Trans.* **2009**, 0 (40), 8755–8764.
- (16) Yamazaki, H.; Ueno, T.; Aiso, K.; Hirahara, M.; Aoki, T.; Nagata, T.; Igarashi, S.; Yagi, M. *Polyhedron* **2013**, 52, 455–460.
- (17) Leto, D. F.; Chattopadhyay, S.; Day, V. W.; Jackson, T. A. *Dalton. Trans.* **2013**, 42 (36), 13014–13025.
- (18) Diril, H.; Chang, H. R.; Zhang, X.; Larsen, S. K.; Potenza, J. A.; Pierpont, C. G.; Schugar, H. J.; Isied, S. S.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1987**, 109 (20), 6207–6208.
- (19) Dubois, L.; Xiang, D. F.; Tan, X. S.; Pécaut, J.; Jones, P.; Baudron, S.; Le Pape, L.; Latour, J. M.; Baffert, C.; Chardon-Noblat, S.; Collomb, M. N.; Deronzier, A. *Inorg. Chem.* **2003**, 42 (3), 750–760.
- (20) Teutloff, C.; Schäfer, K. O.; Sinnecker, S.; Barynin, V.; Bittl, R.; Wieghardt, K.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **2005**, 43 (SPEC. ISS.), 51–64.
- (21) Goodson, P. A.; Hodgson, D. J.; Glerup, J.; Michelsen, K.; Pedersen, E. *Inorg. Chem.* **1990**, 29 (3), 503–508.
- (22) Messinger, J.; Robblee, J. H.; Yu, W. O.; Sauer, K.; Yachandra, V. K.; Klein, M. P. *J. Am. Chem. Soc.* **1997**, 119 (46), 11349–11350.
- (23) Dismukes, G. C.; Sheats, J. E.; Smeel, J. A. *J. Am. Chem. Soc.* **1987**, 109 (23), 7202–7203.
- (24) Schäfer, K. O.; Bittl, R.; Zwegart, W.; Lendzian, F.; Haselhorst, G.; Weyhermüller, T.; Wieghardt, K.; Lubitz, W. *J. Am. Chem. Soc.* **1998**, 120 (50), 13104–13120.
- (25) Mahapatra, S.; Das, P.; Mukherjee, R. *Dalton. Trans.* **1993**, No. 2, 217–220.
- (26) Lessa, J. A.; Horn, A.; Bull, É. S.; Rocha, M. R.; Benassi, M.; Catharino, R. R.; Eberlin, M. N.; Casellato, A.; Noble, C. J.; Hanson, G. R.; Schenk, G.; Silva, G. C.; Antunes, O. A. C.; Fernandes, C. *Inorg. Chem.* **2009**, 48 (10), 4569–4579.

- (27) Hureau, C.; Sabater, L.; Anxolabéhère-Mallart, E.; Nierlich, M.; Charlot, M. F.; Gonnet, F.; Rivière, E.; Blondin, G. *Chem. - A Eur. J.* **2004**, *10* (8), 1998–2010.
- (28) Howard, T.; Telser, J.; DeRose, V. J. *Inorg. Chem.* **2000**, *39* (15), 3379–3385.
- (29) Golombek, A. P.; Hendrich, M. P. *J. Magn. Reson.* **2003**, *165* (1), 33–48.
- (30) Rose, H. R.; Ghosh, M. K.; Maggiolo, A. O.; Pollock, C. J.; Blaesi, E. J.; Hajj, V.; Wei, Y.; Rajakovich, L. J.; Chang, W. C.; Han, Y.; Hajj, M.; Krebs, C.; Silakov, A.; Pandelia, M. E.; Bollinger, J. M.; Boal, A. K. *Biochemistry* **2018**, *57* (18), 2679–2693.
- (31) Schreiber, R. E.; Cohen, H.; Leitun, G.; Wolf, S. G.; Zhou, A.; Que, L.; Neumann, R. *J. Am. Chem. Soc.* **2015**, *137* (27), 8738–8748.
- (32) Stemmler, T. L.; Sossong, T. M.; Goldstein, J. I.; Ash, D. E.; Elgren, T. E.; Kurtz, D. M.; Penner-Hahn, J. E. *Biochemistry* **1997**, *36* (32), 9847–9858.
- (33) Stemmler, T. L.; Sturgeon, B. E.; Randall, D. W.; Britt, R. D.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **1997**, *119* (39), 9215–9225.
- (34) Baldwin, M. J.; Stemmler, T. L.; Riggs-Gelasco, P. J.; James, E. P. H.; Pecoraro, V. L.; Kirk, M. L. *J. Am. Chem. Soc.* **1994**, *116* (25), 11349–11356.
- (35) Banerjee, S.; Draksharapu, A.; Crossland, P. M.; Fan, R.; Guo, Y.; Swart, M.; Que, L. *J. Am. Chem. Soc.* **2020**, *142* (9), 4285–4297.
- (36) Lee, C. M.; Sankaralingam, M.; Chuo, C. H.; Tseng, T. H.; Chen, P. P. Y.; Chiang, M. H.; Li, X. X.; Lee, Y. M.; Nam, W. *Dalton. Trans.* **2019**, *48* (16), 5203–5213.
- (37) Spedalotto, G.; Lovisari, M.; McDonald, A. R. *ACS Omega* **2021**, *6* (42), 28162–28170.
- (38) Cotruvo, J. A.; Stubbe, J. *Biochemistry* **2010**, *49* (6), 1297–1309.
- (39) Costentin, C.; Robert, M.; Savéant, J. M. *J. Am. Chem. Soc.* **2007**, *129* (18), 5870–5879.
- (40) Costentin, C.; Robert, M.; Savéant, J. M.; Teillout, A. L. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (29), 11829–11836.
- (41) Warren, J. J.; Tronic, T. A.; Mayer, J. M. *Chem. Rev.* **2010**, *110* (12), 6961–7001.
- (42) Yan Poon, P. C.; Dedushko, M. A.; Sun, X.; Yang, G.; Toledo, S.; Hayes, E. C.; Johansen, A.; Piquette, M. C.; Rees, J. A.; Stoll, S.; Rybak-Akimova, E.; Kovacs, J. A. *J. Am. Chem. Soc.* **2019**, *141* (38), 15046–15057.
- (43) Lee, Y.; Jackson, T. A. *ChemistrySelect* **2018**, *3* (47), 13507–13516.
- (44) Kappus, H. *Arch. Toxicol.* **1987**, *60* (1–3), 144–149.
- (45) Shook, R. L.; Borovik, A. S. *Inorg. Chem.* **2010**, *49* (8), 3646–3660.
- (46) Lawton, T. J.; Rosenzweig, A. C. *J. Am. Chem. Soc.* **2016**, *138* (30), 9327–9340.
- (47) Rice, D. B.; Wijeratne, G. B.; Burr, A. D.; Parham, J. D.; Day, V. W.; Jackson, T. A. *Inorg. Chem.* **2016**, *55*, 8120.
- (48) Coggins, M. K.; Brines, L. M.; Kovacs, J. A. *Inorg. Chem.* **2013**, *52* (21), 12383–12393.
- (49) Lovisari, M.; McDonald, A. R. *Inorg. Chem.* **2020**, *59* (6), 3659–3665.
- (50) Wijeratne, G. B.; Corzine, B.; Day, V. W.; Jackson, T. A. *Inorg. Chem.* **2014**, *53* (14), 7622–7634.
- (51) Borovik, A. S.; Que, L.; Papaefthymiou, V.; Taylor, L. F.; Anderson, O. P. *J. Am. Chem. Soc.* **1989**, *111* (16), 6183–6195.
- (52) Raycroft, M. A. R.; Maxwell, C. I.; Oldham, R. A. A.; Andrea, A. S.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2012**, *51* (19), 10325–10333.
- (53) Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, *39* (16), 3526–3536.
- (54) Suzuki, M.; Mikuriya, M.; Murata, S.; Uehara, A.; Oshio, H.; Kida, S.; Saito, K. *Bull. Chem. Soc. Jpn.* **1987**, *60* (12), 4305–4312.

- (55) Stoll, S.; Schweiger, A. *J. Magn. Reson.* **2006**, *178* (1), 42–55.
- (56) Ravel, B.; Newville, M. *J. Synchrotron Radiat.* **2005**, *12* (4), 537–541.
- (57) Wojdyr, M. *J. Appl. Crystallogr.* **2010**, *43* (5), 1126–1128.
- (58) Westre, T. E.; Kennepohl, P.; DeWitt, J. G.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1997**, *119* (27), 6297–6314.
- (59) Porter, T. R.; Capita, D.; Kaminsky, W.; Qian, Z.; Mayer, J. M. *Inorg. Chem.* **2016**, *55*, 45.
- (60) Nelsen, S. F.; Kessel, C. R.; Brien, D. J. *J. Am. Chem. Soc.* **1980**, *102* (2).
- (61) Donoghue, P. J.; Gupta, A. K.; Boyce, D. W.; Cramer, C. J.; Tolman, W. B. *J. Am. Chem. Soc.* **2010**, *132* (45), 15869–15871.
- (62) Wittman, J. M.; Hayoun, R.; Kaminsky, W.; Coggins, M. K.; Mayer, J. M. *J. Am. Chem. Soc.* **2013**, *135* (35), 12956–12959.

Chapter 4: Thermal Activation of a Mn^{II}Mn^{III}-Peroxide and the Mechanistic Implications for the Catalytic Cycle of Ib RNRs

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4.0 Abstract

Class Ib ribonucleotide reductase (RNR) catalyse the conversion of ribonucleotides to the corresponding deoxyribonucleotides. Many questions still surround the mechanism of Ib RNRs due to the short lifetimes of key intermediates which makes trapping and analysis of them difficult. Using the small molecule mimic of the active site $[\text{Mn}^{\text{II}}_2(\text{BPMP})(\text{OCCH}_3)](\text{ClO}_4)$ (**1**), we have replicated the structure and activity of Ib RNRs.

The reaction of **1** with superoxide resulted in the formation of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**). The thermal decay product of **2** was analysed via UV-vis, EPR, X-ray absorption spectroscopies, and mass spectrometry and determined to form a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (**5**), similar to the implicated active oxidant in the catalytic cycle of Ib RNRs. New species **5** was found to react with a variety of *para* substituted 2,6-di-*tert*-butylphenols to generate the corresponding phenoxyl radical species by EPR. Kinetic analysis of **5** reactivity with substrates found a non-linear trend that was modelled as Michaelis-Menten kinetics. We postulated the origin of these non-linear kinetics was due to an activation of **5** towards oxidation after coordination of the O-H substrate to **5**.

This work mimicked the observed chemistry in Ib RNRs whereby a Mn^{II}_2 active site reacted with superoxide to form a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species and oxidised an O-H bond containing substrate to form a phenoxyl radical. This work not only provided support to the postulated catalytic cycle in Ib RNRs, but supported a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide as the unknown species that preceded $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ formation.

4.1 Introduction

Ribonucleotide reductase (RNRs) are metalloenzymes that catalyse the conversion of ribonucleotides to the corresponding deoxyribonucleotides, representing the initial and rate limiting step of DNA synthesis and repair in all organisms.¹ In the proposed mechanism for the manganese containing subclass Ib RNRs; superoxide (O_2^-) is generated via interaction of O_2 with a nearby flavodoxin protein (Nrd_{hq}).² O_2^- reacted with the Mn^{II}_2 active site to form an unknown intermediary species that rapidly converted to create a bis(μ -oxo) $Mn^{III}Mn^{IV}$ species (Figure 4.1).³ This $Mn^{III}Mn^{IV}$ was responsible for oxidation of the tyrosine residue to form a tyrosyl radical that initiated the ribonucleotide reduction.

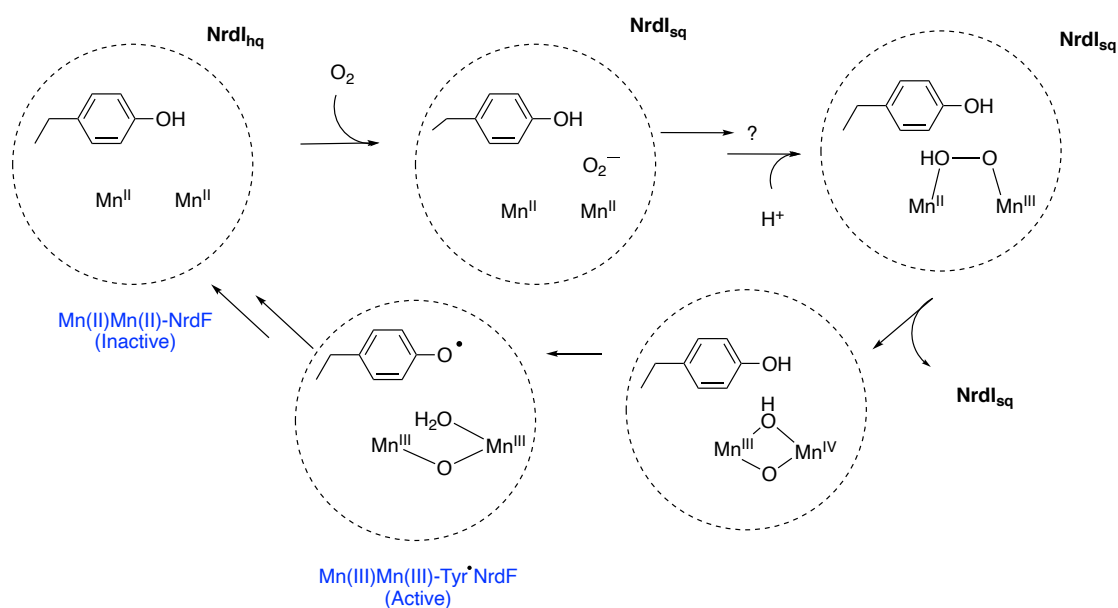


Figure 4.1: Proposed mechanism for Class Ib RNRs.

Due to the short lifetimes and rapid conversion to the $Mn^{III}Mn^{IV}$ species, the unknown intermediary that followed reaction of O_2^- with Mn^{II}_2 has never been trapped and analysed via spectroscopic techniques. Therefore, no experimental evidence exists for its structure. We postulate that the identity of this unknown species could be a $Mn^{II}Mn^{III}$ -peroxide species as mixed valent peroxides were generated following reaction of O_2^- with small

molecule mimics of the active site of Ib RNRs.^{4,5} We are interested in replicating the next step of this cycle and attempting to generate a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species from the peroxo- $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$. Interestingly, bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species have been found to be the thermal decay products of Mn^{III} -peroxides.^{6,7} We therefore were interested in activating the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species **2** via warming to achieve a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ adduct whose reactivity we could probe to understand more about the tyrosine oxidation step in class Ib RNRs.

4.2 Results and Discussion

A 1.5 mM solution of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species **2** (Chapter 2) in 1:9 acetonitrile(MeCN)/tetrahydrofuran(THF) was warmed from $-90\text{ }^{\circ}\text{C}$ to $+20\text{ }^{\circ}\text{C}$ over 900 seconds. This resulted in a change from the distinctive and defined UV-vis absorption features of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** to form a new broad, featureless absorption associated with the conversion to a new species, (**5**) (Figure 4.2). New species **5** was found to be stable at $+20\text{ }^{\circ}\text{C}$ with a half-life of $t_{1/2} = 2310$ seconds. This type of broad UV-vis spectrum lacking any key distinctive absorption bands was not typical of any of the common dinuclear manganese products, which all generally displayed some absorbance features, typically derived from ligand to metal charge transfer or $d-d$ -transitions.^{8,9} We postulated that the heating of **2** would result in the formation of a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex as these species have been observed before as products of the thermal decay of Mn^{III} -peroxides.^{6,7} Typically $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species displayed an intense absorption feature between 500-700 nm associated with a $\text{O} \rightarrow \text{Mn}^{\text{IV}}$ charge transfer.⁸ However, there was precedent for such features displayed by **5** in the literature of $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes. For example, the complex $[\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(2\text{-OH}(3,5\text{-Cl}_2\text{Sal})\text{pn})_2]\text{ClO}_4$ reported by Pecoraro and co-workers also showed a broad featureless spectrum that tailed into the UV-region.¹⁰ Even more relevant was the UV-vis spectrum of a putative $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species that also displayed a similar tailing absorption into the UV region in the recently discovered class Id RNRs.¹¹ Therefore, new species **5** could be new $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ with an unconventional, though not unprecedented, electronic absorption spectrum.

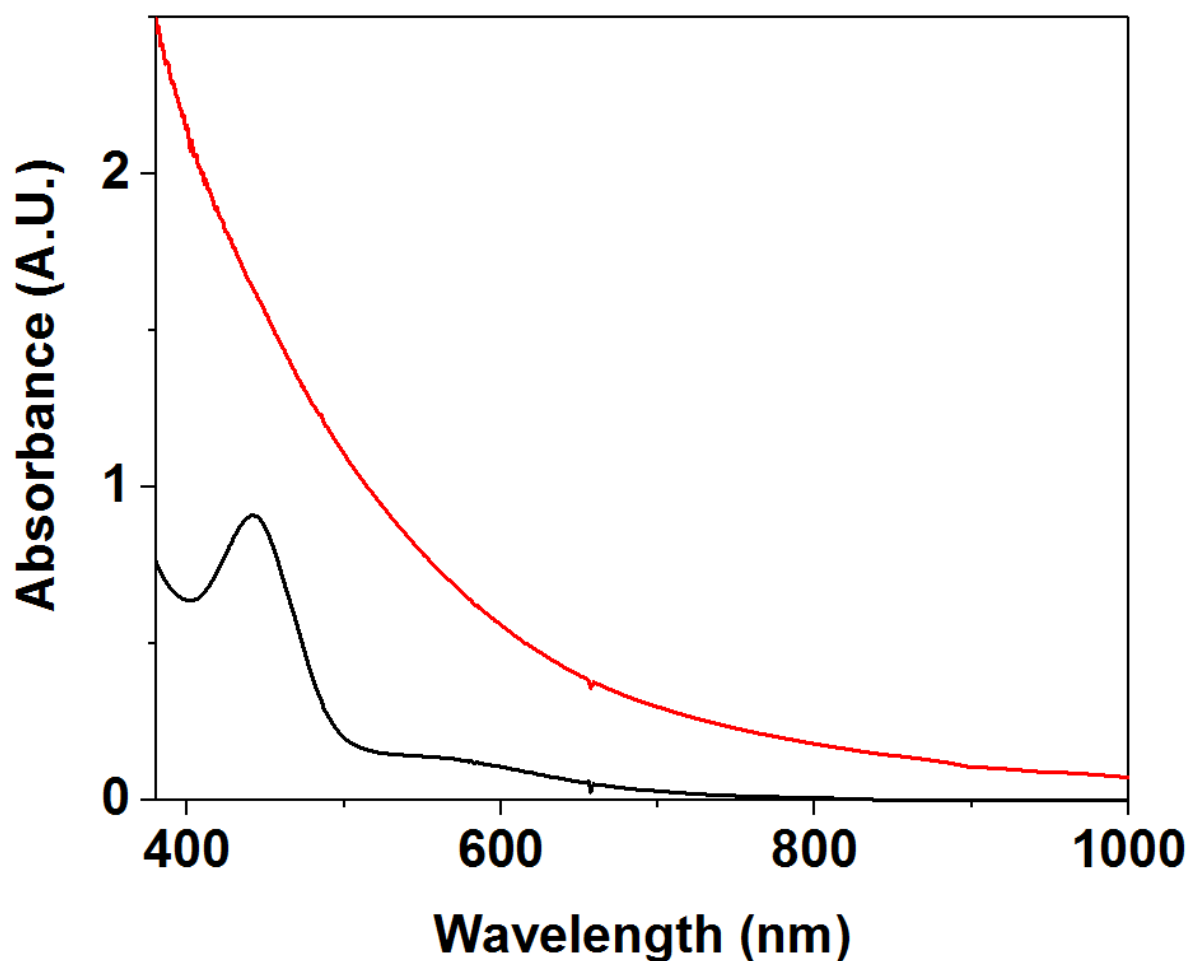


Figure 4.2: Electronic absorption features of the reaction of **2** (black trace) during warming from -90°C to $+20^{\circ}\text{C}$ to form new species **5** (red trace) in 1:9 MeCN/THF.

EPR spectroscopy was performed on frozen samples of **5** in order to investigate the oxidation states of the Mn centres. EPR analysis of **5** measured at 2 K revealed two new signals (Figure 4.3, red trace). A new 11-line species centred at $g = 2.6$ and a 16-line signal at $g = 2$. EPR measurements of a frozen sample of **5** were also performed at 30 K (Figure 4.3, blue trace). At 30 K, the two signals can still be observed; a 16-line signal centred at $g = 2$ and an 11-line signal at $g = 2.6$ (Figure 4.3, blue trace). Both species displayed a much narrower line and spectral widths than the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide precursor species, **2**, also shown in Figure 4.3 (black trace) which indicated that both of the new signals originated from a new species.

In both the 2 K and 30 K spectra the 16-line signal was centred at $g = 2$ and displayed a

spectral width of 120 mT and a hyperfine coupling constant (A) of $A_{av} = 7.9$ mT. A 16-line signal at $g = 2$ was characteristic of the formation of a bis(μ -oxo) $Mn^{III}Mn^{IV}$ complex, which due to the strong antiferromagnetic coupling between the two metal centres, resulted in a superposition of the hyperfine lines and produced the distinctive 16-line signal.¹² The A_{av} of the 16-line signal was calculated to be 7.9 mT and was consistent with previously reported $Mn^{III}Mn^{IV}$ species. For example, a bis(μ -oxo) $Mn^{III}Mn^{IV}$ species presented by Pedersen and co-workers reported an A -value of 7.8 mT.¹³ Moreover, the narrow spectra width of the $g = 2$ signal of just 120 mT was approximately half that of 250 mT width of the $Mn^{II}Mn^{III}$ -peroxide **2**. Typically, bis(μ -oxo) $Mn^{III}Mn^{IV}$ complexes displayed much narrower spectral widths in comparison to $Mn^{II}Mn^{III}$, as the effective hyperfine tensors of $Mn^{II}Mn^{III}$ complexes are larger than $Mn^{III}Mn^{IV}$ species and resulted in broader spectral widths.^{14,15} In fact, Dismukes and co-workers suggested that the larger spectral width of $Mn^{II}Mn^{III}$ complexes compared to $Mn^{III}Mn^{IV}$ ones could be used as a means to differentiate the two.¹⁶ Finally, the temperature independence of the signal at $g = 2$ was also consistent with a bis(μ -oxo) $Mn^{III}Mn^{IV}$, as these complexes have been measured at up to 77 K, while $Mn^{II}Mn^{III}$ systems must be recorded at temperatures <15 K in order to resolve the signal.¹⁷ Therefore, we assigned **5** to be a bis(μ -oxo) $Mn^{III}Mn^{IV}$ complex. The yield of the 16-line species was determined by EPR integration to be 60 % $\pm$ 15 % (A4.1).

One other signal was observed in the EPR of **5**; an 11-line signal at $g = 2.6$ displayed a spectral width of 45 mT and $A_{av} = 4.4$ mT. The 11 hyperfine lines indicated that this species contained two equivalent manganese centres $\{(2nI+1) = (2(2)(5/2)+1)) = 11\}$. The 11-line signal agreed well with that observed for previously reported Mn^{II}_2 complexes, such as $[Mn^{II}_2(TMEDA)_2(OCCH_3)_4(H_2O)]$ and $[Mn^{II}_2(Me_3TACN)_2(\mu-OCCH_3)_3]BPh_4$.^{18,19} The A_{av} values of $[Mn^{II}_2(TMEDA)_2(OCCH_3)_4(H_2O)]$ and $[Mn^{II}_2(Me_3TACN)_2(\mu-OCCH_3)_3]BPh_4$ were calculated to be 4.5 and 4.6 mT respectively, once again in agreement with that of 4.4

mT measured for the 11-line signal.^{18,19} Therefore, the 11-line signal was determined to have originated from a Mn^{II}_2 complex. The yield of the 11-line species was found to be <10% via EPR integration. Therefore, we assigned the 11-line signal to have originated from a Mn^{II}_2 complex. EPR studies of the Mn^{II}_2 starting material **1**, revealed an EPR silent spectrum, indicating that this complex was not the source of the $g = 2.6$ signal. Variable temperature EPR studies of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species **2** revealed an 11-line signal at $g = 2.6$ that appeared when **2** was recorded >30 K (A4.2). Therefore, we postulated that this 11-line species was a side-product formed during the reaction of **1** and O_2^- .

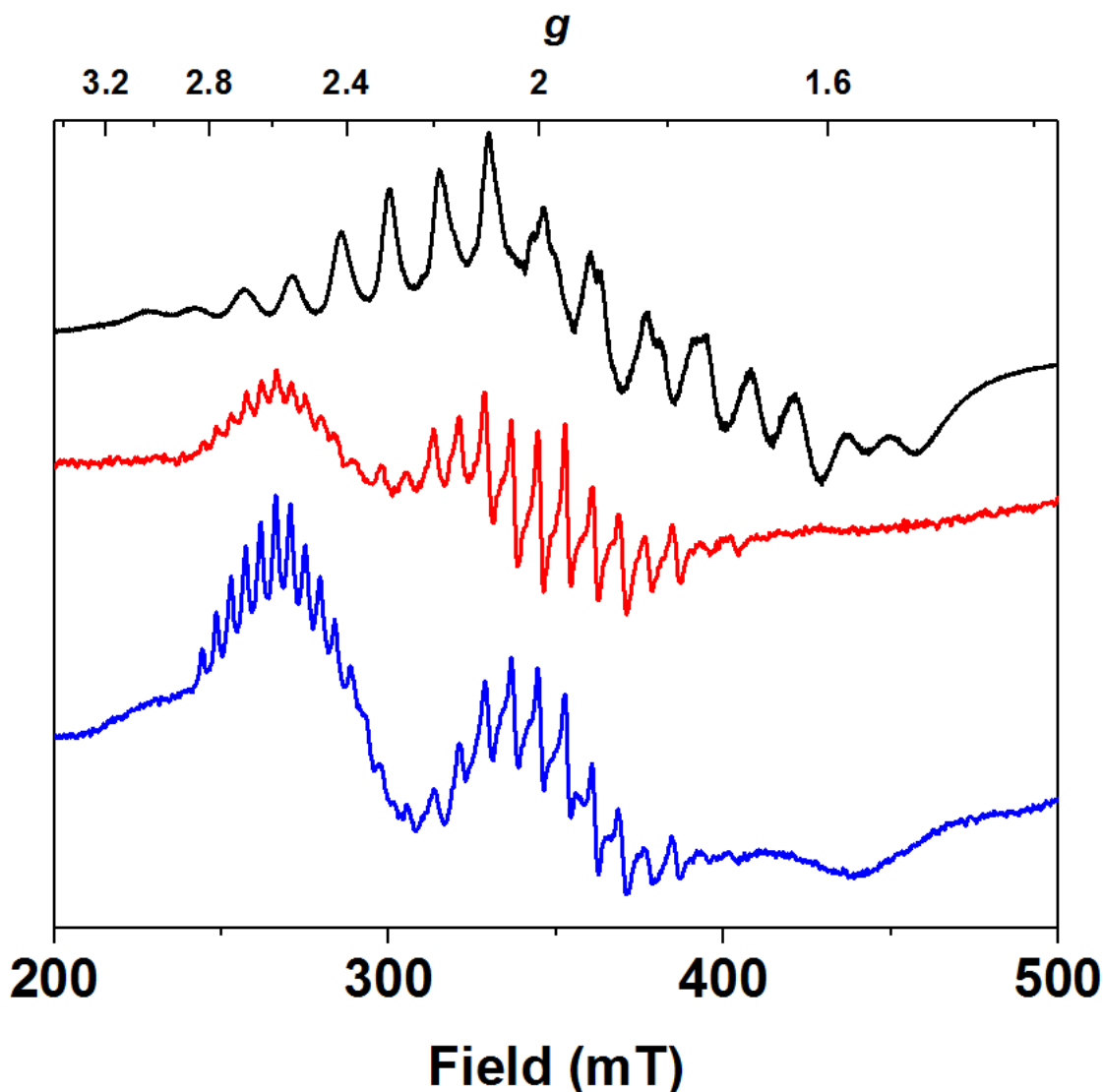


Figure 4.3: EPR spectra of **2** recorded at 2 K (black trace), **5** recorded at 2 K (red trace), and **5** recorded at 30 K (blue trace). (9.65 GHz, 0.2 mW microwave power)

Interestingly, the EPR features of **5** recorded at 30 K, were very similar to that of the bis(μ -oxo)(μ -hydroxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ (**4**) studied in chapter 3 (Figure 4.4). The EPR spectrum of **4** recorded at 30 K displayed two features; an 11-line signal centred at $g = 2.6$ with $A_{av} =$ and a 16-line signal centred at $g = 2$ with $A_{av} = 7.4$ mT. Very similar to the spectrum of **5** at 30 K that also displayed two signals at 30 K; a 11-line signal centred at $g = 2.6$ with $A_{av} =$ and a 16-line signal centred at $g = 2$ with $A_{av} = 7.8$ mT. In both the spectra of **4** and **5** an 11-line signal was observed centred around $g = 2.6$ with $A_{av} = 4.4$ mT. This was interesting, as in

both the spectrum of **4** and **5**, we postulated the 11-line species originated from a Mn^{II}_2 side-product formed during the reaction of O_2^- and **1**. This was evidenced by the formation of the same 11-line signal in the EPR spectrum of **2** measured at 32 K. Both complexes **4** and **5** are generated from **2** and therefore the same Mn^{II}_2 must have formed in both reactions to generate the 11-line signal in both spectra.

Interestingly, in the spectra of **4** and **5**, the signals at $g = 2$ shared a number of similarities (Figure 4.4); Both signals displayed a 16-line hyperfine pattern centred at $g = 2$. The signals displayed a similar narrow width of 125 and 110 mT for **4** and **5** respectively. Finally, the 16-line signals from **4** and **5** were resolved at EPR measurement temperatures of 30 K. The number of parallels between the complexes and **4** and **5** may indicate that they had formed similar species.

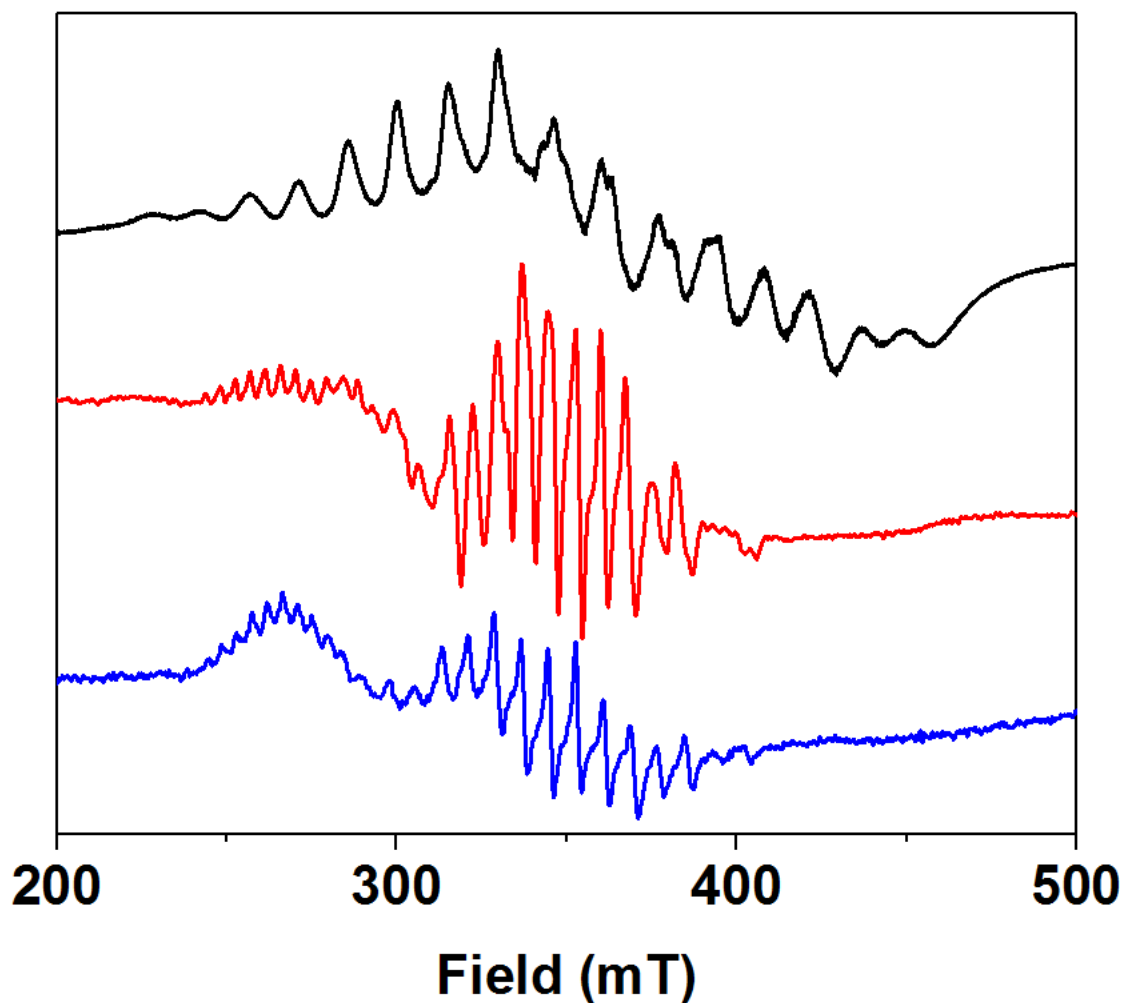


Figure 4.4: EPR spectra of **2** (black trace) recorded at 2 K, **4** (red trace) recorded at 7 K, and **5** (blue trace) recorded at 30 K (9.65 GHz, 0.2 mW microwave power).

Cold injection electrospray ionisation mass spectrometry (ESI-MS) of a just-thawed methanol solution of **5** revealed a peak at $m/z = 335.5808$ that agreed with the calculated peak of $m/z = 335.5682$ for the dication $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}\text{bis}(\mu\text{-oxo})(\text{BPMP})]^{2+}$ (Figure 4.5, left). This peak was not observed in the mass spectrum of **2** (A4.4). The separation of the peaks was calculated to be 0.5 units, consistent with the assignment of a dication species. Complex **5** was prepared using K^{18}O_2 and resulted in the peak assigned to $[\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}\text{bis}(\mu\text{-oxo})(\text{BPMP})]^{2+}$ to shift by two mass unit to $m/z = 337.5788$, rationalised as the incorporation of two ^{18}O oxygen atoms into the oxo moiety in a dication molecule. This was further indicative that **5** was forming an oxo-bridged $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species.

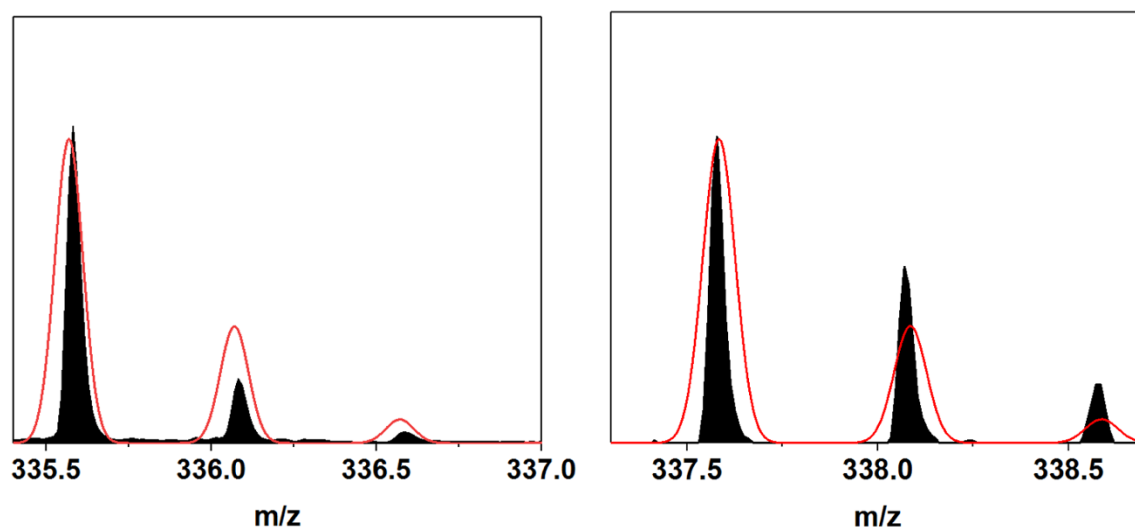


Figure 4.5: ESI-MS spectra of **5** prepared using $K^{16}O_2$ (left) and $K^{18}O_2$ (right). Insets: Simulated mass spectra.

In order to analyse the oxidation states and local geometries of the Mn centres in complex **5**, Mn K-edge X-ray absorption near edge spectroscopy (XANES) was performed (Figure 4.6). The XANES spectra of **5** displayed a rising edge with first inflection point calculated to be at 6549.0 eV (Figure 4.6, red trace). The XANES spectra of **5** also contained a pre-edge peak that was fit using two 50:50 pseudo-Voigt functions and determined that the peak was centred at 6540.5 eV with an area of 8.7 units (A4.6). By comparison, the $Mn^{II}Mn^{III}$ -peroxide species **2**, the precursor to **5**, had a rising edge with first inflection point at 6548.9 eV, a pre-edge peak that was calculated to be centred at 6540.6 eV with an area of 13.5 units (Figure 4.6, black trace).

Comparing the XANES spectra of **2** and **5**, one of the most noticeable difference was a decrease in the intensity of shoulder at ~ 6552 eV in **5**. This shoulder feature has been observed previously in the XANES spectra of Id RNRs, and represented Mn^{II}_2 species.¹¹ Therefore, it was inferred there was less Mn^{II}_2 in sample **5** compared to **2**, which indicated the conversion of **2** to a new species. Upon conversion of **2** to **5**, there was a shift in the

edge energy from 6548.9 eV to 6549.0 eV respectively, representing a change of just 0.1 eV (Table 4.1). Typically in the XANES spectra of Mn complexes, an increase in the formal oxidation state of a Mn centre by +1 was associated with an edge energy shift of between 2 – 4 eV.^{20,21} Therefore, a shift of just 0.1 eV was smaller than what we would have expected for the conversion of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ to a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. However, as XANES is a measure of the average oxidation state of all Mn centres in the sample, it could be possible that the Mn^{II}_2 observed in the EPR spectra of **5** resulted in an “averaging down” of the edge energy.²² Furthermore, Mn samples are susceptible to photoreduction from the X-rays during XANES spectroscopy.^{1,23} This may have occurred during measurement of sample **5** and effected the observed edge shift.

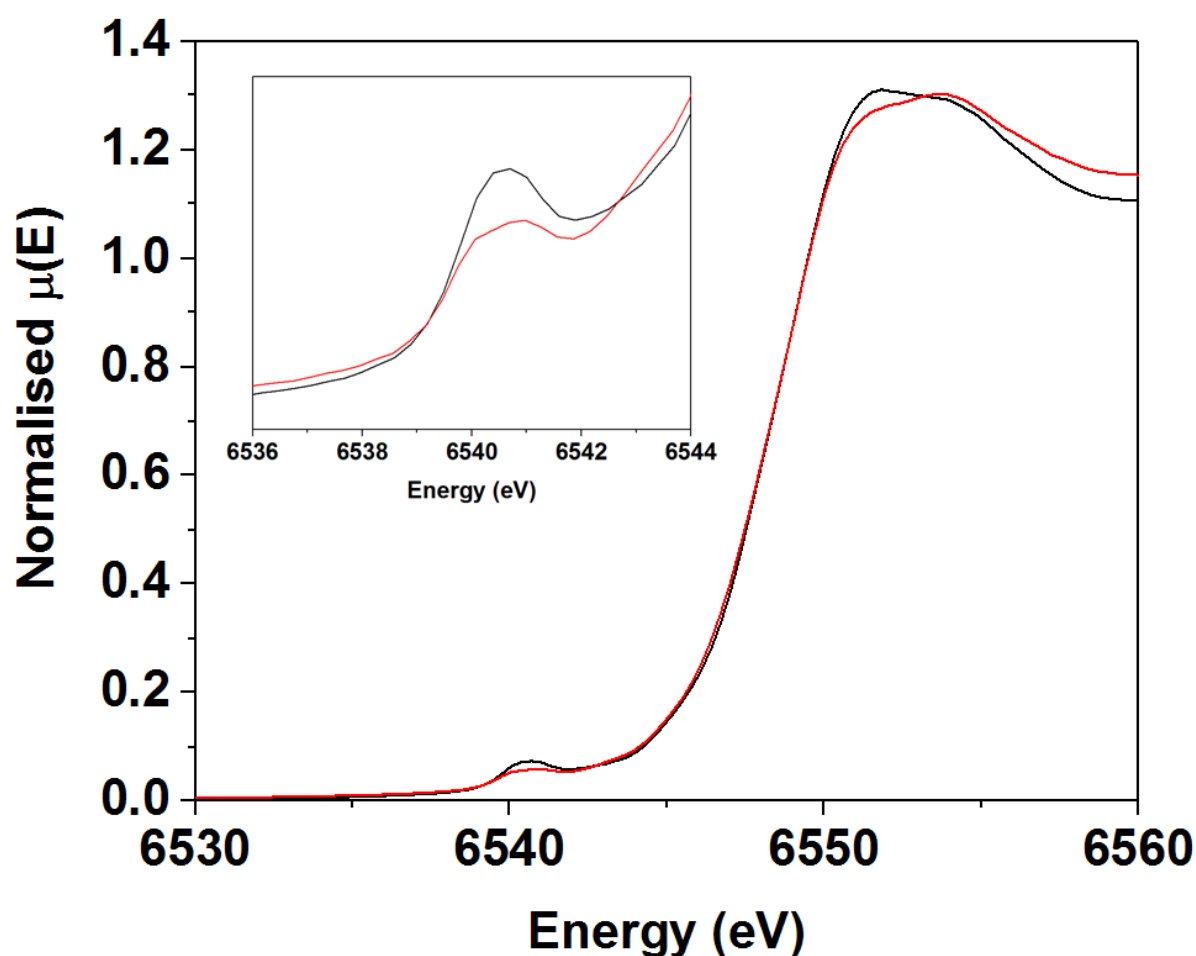


Figure 4.6: Normalised XANES spectra of **2** (black trace) and **5** (red trace). Inset: Expansion of the pre-edge region.

The pre-edge features of **2** and **5** were centred at 6540.6 and 6540.5 eV respectively, representing a shift of 0.1 eV to lower energy (Table 4.1). Interestingly, complex **5** displayed a pre-edge area of 8.7 units, a decrease of 4.8 units in comparison to the pre-edge area of **2**. The pre-edge region of the XANES spectra represented the crystal field transitions from the core 1s to the 3d orbitals.²⁰ A decrease in the pre-edge area was related to an increase in the electronic symmetry around the metal centres. Therefore, the metal centres of **5** had a more symmetric local geometry compared to **2**. This was somewhat unexpected as increases in the Mn oxidation states were associated with increased distortions around the metal centres.²⁰ However, metal centre oxidation states are not the only factor that determines electronic symmetry, local geometry also plays a role. This is easily illustrated in figure 4.7, where the higher symmetry contained in a six coordinate species compared to a five coordinate species can be easily seen. This is further illustrated by the high point group of the six-coordinate complex compared to the five coordinate one. The increase in symmetry moving from the Mn^{II}Mn^{III}-peroxide to a six coordinate bis(μ -oxo)Mn^{III}Mn^{IV} species would result in a smaller pre-edge area.

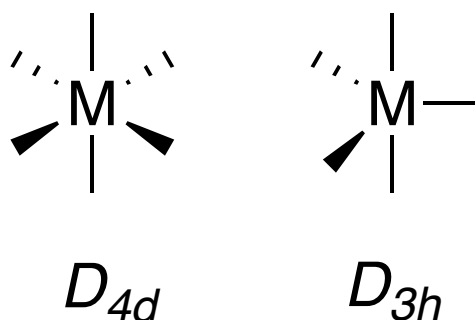


Figure 4.7: Six coordinate versus five coordinate point groups.

Both **5** and the (μ -hydroxo)(μ -oxo)Mn^{III}Mn^{IV} species **4** (Chapter 3) displayed similar XANES spectra. **4** exhibited a rising edge with a first inflection point at 6549.4 eV,

compared to 6549.0 eV for **5** (Table 4.1). This represented an edge energy difference of just 0.4 eV between **4** and **5**. The pre-edge areas of **4** and **5** were also similar. The pre-edge of **4** and **5** were both centred at 6540.5 eV with calculated areas of 10.2 and 8.7 units respectively. Both the edge energies and pre-edge centres of **4** and **5** were in agreement with each other and indicated that the species shared the same oxidation states. Interestingly, the pre-edge area of **4** was greater than that of **5** by 1.5 units. This suggested that the metal centres of **4** contained a less symmetric geometry in comparison to the metal centres of **5**. We postulated that **5** contained a more symmetric bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ structure in comparison to the (μ -hydroxo)(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ structure of **4**. The protonated (μ -hydroxo) bridge in **4** introduced distortion into the oxo core of the complex and made **4** more asymmetric compared to the mirrored geometry of the “naked” bis(μ -oxo) structure of **5**.

Table 4.1: Comparison of the XANES data for complexes **1**, **2**, and **5**.

Complex	Edge Energy (eV)	Pre-Edge Area (units)	Pre-Edge Peak (eV)
1	6548.4 ($\pm$ 0.1)	8.8 ($\pm$ 0.1)	6540.4 ($\pm$ 0.1)
2	6548.9 ($\pm$ 0.1)	13.5 ($\pm$ 0.1)	6540.6 ($\pm$ 0.1)
4	6549.4 ($\pm$ 0.1)	10.2 ($\pm$ 0.1)	6540.5 ($\pm$ 0.1)
5	6549.0 ($\pm$ 0.1)	8.7 ($\pm$ 0.1)	6540.5 ($\pm$ 0.1)

We were interested in further exploring the differences between complexes **4** and **5**. It was found that upon addition of the base triethylamine (NEt_3) to **4**, an immediate bleaching of the spectrum was observed (Figure 4.8, left). Therefore, we were interested in probing the protonation state of **5**. Upon addition of 50 eq. of NEt_3 a small change in the intensity of

the electronic features associated with **5** was observed (Figure 4.8, right). As there was no change in the shape of the absorbance features of **5** this decrease was determined to arise from a dilution effect upon addition of the volume of base. Therefore, we concluded that **5** does not react with the base NEt_3 , however, the $(\mu\text{-hydroxo})(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species **4** does. In fact, the electronic absorption spectra of the new species formed following the reaction of **4** with NEt_3 was similar to the broad, featureless absorption of **5**. We postulated that **4** was deprotonated by the base NEt_3 to form a bis($\mu\text{-oxo}$) species, with a structure similar to **5**.

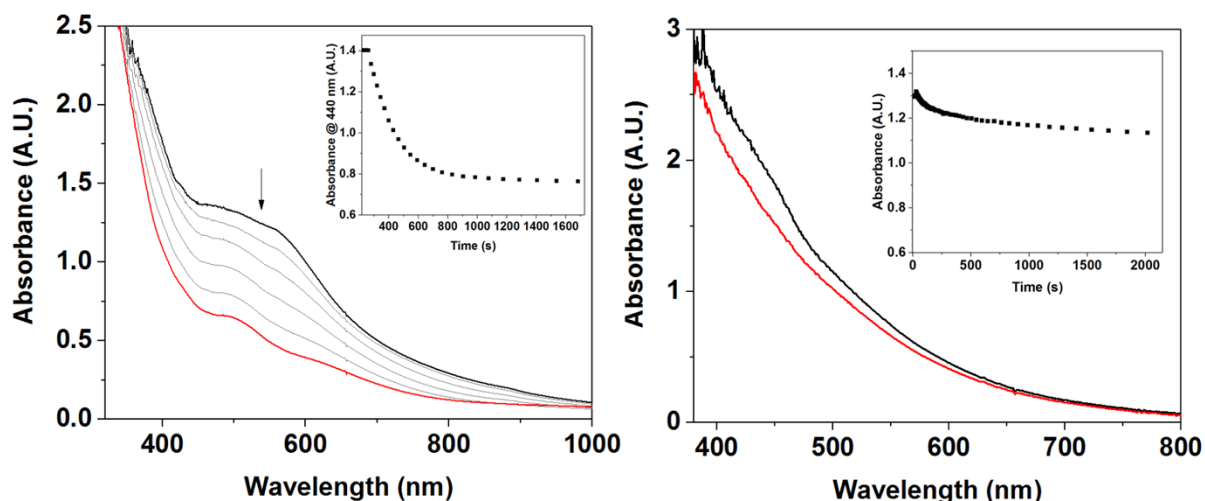


Figure 4.8: Left: electronic absorption features of the reaction of **4** (black trace) + 50 eq. NEt_3 (red trace). Inset: plot of the absorbance at 440 nm versus time during reaction. Right: electronic absorption features of the reaction of **5** (black trace) + 50 eq. NEt_3 (red trace). Inset: plot of the absorbance at 440 nm versus time during reaction.

With the combination of UV-vis, EPR, X-ray absorption spectroscopies and mass spectrometry data, we assigned the new species **5** formed following the thermal decay of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** to be a new bis($\mu\text{-oxo}$) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex (Figure 4.9). In analogy to the formation of **5** from **2**, Jackson *et al.* reported a Mn^{III} -peroxide $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{N4py})]^{2+}$ that thermally decayed to form a bis($\mu\text{-oxo}$) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species $[\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}(\text{N4py})_2]^{3+}$.⁶ Jackson *et al.* speculated that the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ was formed following the generation of a dinuclear peroxo-bridge intermediate $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\mu\text{-O}_2)(\kappa^4\text{-$

N4py)₂]³⁺, similar to **2**. It was postulated that both Mn centres would provide an electron to cleave the O-O and resulted in formation of the bis(μ -oxo)Mn^{III}Mn^{IV} complex. However, they were unable to provide experimental evidence for the dinuclear peroxide species. We postulated that the bis(μ -oxo)Mn^{III}Mn^{IV} complex **5** was formed following the O-O bond cleavage of the peroxide in **2** following thermal activation.

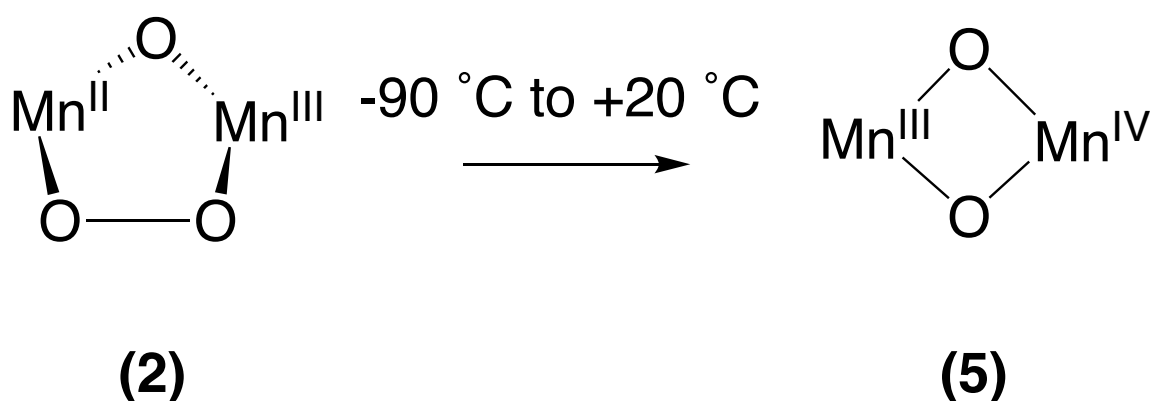


Figure 4.9: Conversion from **2** to **5** upon heating from -90 °C to +20 °C.

4.3 Reactivity with Phenols

Following the formation of the new Mn^{III}Mn^{IV} species (**5**) we were interested in seeing if this new complex could also mimic the reactivity observed in Ib RNRs. In the catalytic cycle proposed by Stubbe; a Mn^{III}Mn^{IV} species reacted with a tyrosine to generate a tyrosyl radical, which then initiated ribonucleotide reduction.³ 4-methoxy-2,6-di-*tert*-butylphenol (4-OCH₃-2,6-DTBP) was chosen to act as a functional mimic for the tyrosine residue and to probe the reactivity of **5**. Upon addition of 10 eq. of 4-OCH₃-2,6-DTBP, a decrease in the absorption features associated with **5** was observed with the simultaneous increase in a new feature at $\lambda_{\text{max}} = 405$ nm. This sharp peak was commonly associated with the formation of the phenoxyl radical species.²⁴ To further investigate this, the 4-methoxy-2,6-di-*tert*-butylphenoxyl radical was independently synthesised and its electronic absorption spectrum in the same measurement conditions also revealed an intense sharp peak at λ_{max}

= 405 nm (A4.7). Attempts to determine a yield for the radical were unsuccessful as the radical was unstable in the solvent mixture and began decaying rapidly once dissolved (A4.8). Therefore, an accurate absorption coefficient could not be calculated.

A frozen EPR sample of the post-reaction mixture was also measured. A sharp, intense signal centred at $g = 2$ was recorded, strongly indicating the formation of the corresponding organic radical for 4-OCH₃-2,6-DTBP (Figure 4.10). The yield of the 2,6-di-*tert*-butyl-4-methoxy-phenoxy radical was determined to be $75 \pm 15\%$ by EPR when a one-electron reaction was considered. It was proposed that the reaction between the Mn^{III}Mn^{IV} species **5** with the tyrosine mimic 4-OCH₃-2,6-DTBP, resulted in the formation of the corresponding phenoxy radical. This was in analogy with the reaction of the Mn^{III}Mn^{IV} species in Ib RNRs that oxidised the phenol O-H bond of the tyrosine residue to a tyrosyl radical.

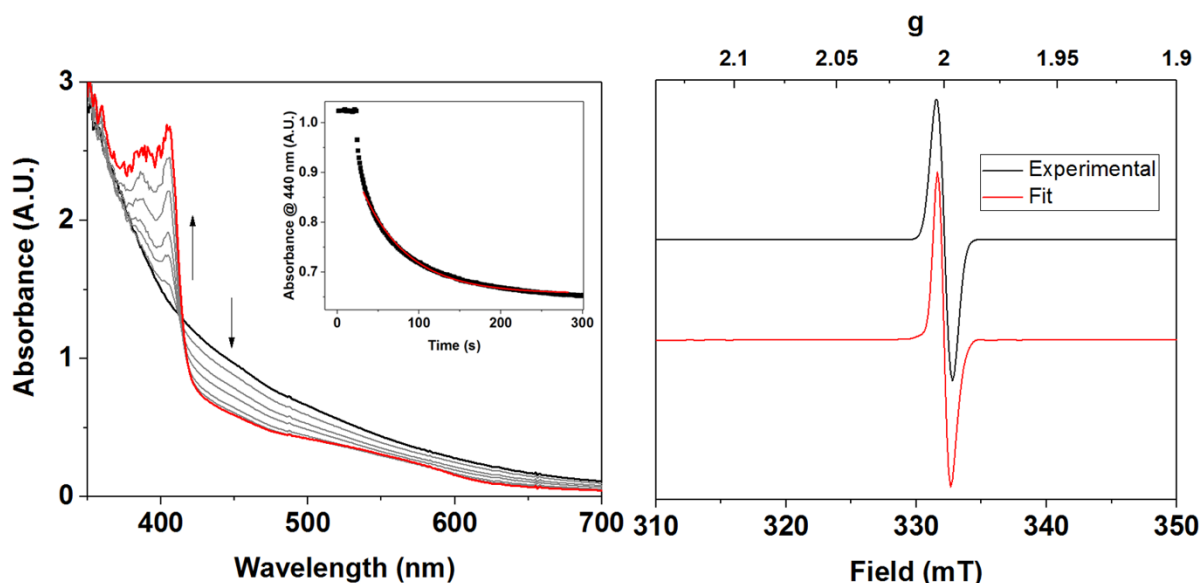


Figure 4.10: Left: Electronic absorption spectra change of the reaction of **5** with 10 eq. 2,6-di-*tert*-butyl-4-methoxyphenol. Inset: Plot of the change of absorption at 550 nm over time fitted as a pseudo first-order reaction. Right: Perpendicular X-Band EPR spectrum of the reaction of **5** with 4-OCH₃-2,6-DTBP measured at 77 K (9.65 GHz, 0.2 mW microwave power) and the simulated spectra (right).

Inspired by **5**'s reactivity with 4-OCH₃-2,6-DTBP, we decided to further investigate the kinetics of the complex using 4-substituted-2,6-di-*tert*-butylphenols. **5** was reacted with

phenols 4-X-2,6-DTBP where X = (OCH₃, CN, Me, H, C₂H₅, and C(CH₃)₃). In the reaction of **5** with varying concentrations of the substituted phenols the change in absorbance at $\lambda_{max} = 440$ nm in the electronic absorption spectra was plotted versus time. Based on this plot, a *pseudo* first-order rate constant (k_{obs}) for the reaction was determined by fitting the resulting curve (A4.9). For the 4-H-2,6-DTBP substrate, a product was formed that masked the decay of **5** at $\lambda_{max} = 440$ nm, hence, for this substrate the decay of **5** was monitored using $\lambda_{max} = 550$ nm (A4.14 and A4.15). Plotting the k_{obs} versus substrate concentration ([S]) revealed a non-linear saturation behaviour commonly associated with enzyme kinetics (Figure 4.11).²⁵ This indicated the presence of an equilibrium prior to irreversible substrate transformation.

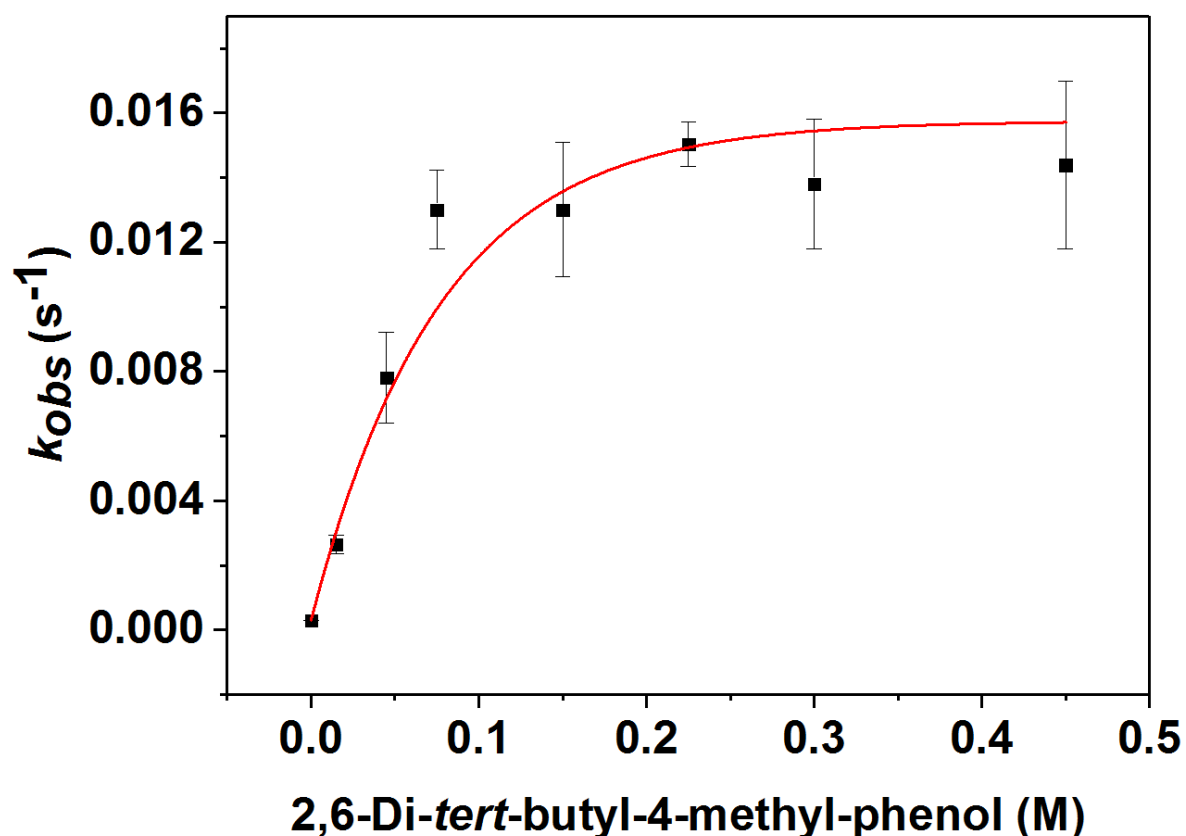


Figure 4.11: Plot of substrate concentration versus k_{obs} for the reaction of **5** and 2,6-di-*tert*-butyl-4-methyl-phenol.

The phenols containing X = Me, H, C₂H₅, and C(CH₃)₃ functionalities displayed Michaelis-Menten type behaviour (A4.10, A4.13, A4.16, A4.19, and A4.21).²⁶ Michaelis-Menten kinetics were associated with a two-step reaction process, where the first step was reversible followed by an irreversible second step (Figure 4.12). At lower concentrations of the substrate, the reversible step determined the reaction rate and as substrate concentration increased so does the rate. As the concentration of the substrate increased further the equilibrium shifted to the forward reaction and the irreversible step was now the rate determining step and lead to the characteristic saturation behaviour observed for Michaelis-Menten kinetics (Figure 4.11).

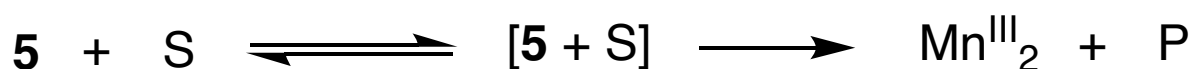


Figure 4.12: Reaction pathway during a Michaelis-Menten type reaction where S = substrate, P = products.

The substrates 4-X-2,6-DTBP where X = OCH₃, Me, H, C₂H₅, and C(CH₃)₃, displayed typical Michaelis-Menten kinetics. An exception to this trend was the substrate 4-CN-2,6-DTBP that showed a Hill type behaviour (A4.24). Hill models are seen in enzymes that display cooperativity (*n*). A value of *n* > 1 in a system displaying non-linear *k_{obs}* vs [S] behaviour may be understood as more than one substrate molecule is involved in the reaction.²⁷ The cooperativity for the reaction between **5** and 4-CN-2,6-DTBP was calculated to be 2.95 ± 1. The change from Michaelis-Menten behaviour to a Hill type may be attributed to the increased acidity of the O-H bond of 4-CN-2,6-DTBP. This has been observed previously when a Ni^{III}-fluoride complex exhibited a change in its mechanism of oxidation of *para*-substituted-2,6-di-*tert*-butylphenols as the acidity of the O-H bond increased.²⁸ We postulated that a similar behaviour was observed in the case of **5**.

The post-reaction mixture of **5** with the phenols was analysed via EPR spectroscopy (A4.31 - A4.36). Analysis of 4-H-2,6-DTBP and 4-C(CH₃)₃-2,6-DTBP post-reaction mixture revealed the formation of the corresponding phenoxyl radical species, albeit in much lower yields than that calculated for the phenoxyl radical found after reaction of **5** and 4-OCH₃-2,6-DTBP (Table 4.2). Phenols 4-CH₃-2,6-DTBP and 4-CN-2,6-DTBP displayed an EPR silent spectrum at 77 K, possibility indicating that the radicals formed had taken part in further reactions and resulted in EPR silent products. For 4-X-2,6-DTBP where X = OCH₃, C(CH₃)₃, CN, and C₂H₅, gas chromatography analysis of the post-reaction mixture revealed the formation of 2,6-di-*tert*-butyl-1,4-benzoquinone (2,6-DTBQ) (A4.37 - A4.42).

The intense phenoxyl radical signal displayed in some of the post-reaction mixtures was narrower than the dinuclear manganese based EPR signals observed previously. Despite this, no other signal was observed in the EPR spectrum. Furthermore, the EPR of the post-reaction mixture of all phenols showed no evidence for metal-based signals. Therefore, we proposed that following reaction with 4-OCH₃-2,6-DTBP, **5** converted to an EPR silent Mn^{III}Mn^{III} species. This was similar to what was observed during the catalytic cycle of Ib RNRs, whereby the postulated Mn^{III}Mn^{IV} species reacted with the tyrosine residue to form a Mn^{III}Mn^{III} species which had an integer value of *S* and was therefore not observed in the post reaction EPR spectrum.² Moreover, no metal based EPR signals were observed in the post reaction mixture of **4** and 4-OCH₃-2,6-DTBP (Chapter 3). Therefore, we postulated that both **4** and **5** reacted with the phenol to form similar EPR silent Mn^{III}Mn^{III} products.

4.4 Mechanistic Analysis

Several potential mechanisms can explain the saturation kinetics observed during the reaction of **5** with phenols (Figure 4.13). The saturation behaviour indicated that an initial equilibrium preceded the formation of an intermediate species prior to the rate-determining

generation of a phenoxyl radical and a $\text{Mn}^{\text{III}}\text{Mn}^{\text{III}}$ species.²⁹ One possibility was a reversible proton/electron transfer from the phenol to **5** followed by an irreversible electron/proton transfer (Figure 4.13, Path A/C). Another possible mechanism involved a reversible coordination of the substrate to the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core followed by an irreversible one step oxidation of the phenol to phenoxyl radical via concerted proton coupled electron transfer (CPET) or hydrogen atom transfer (HAT).³⁰ (Figure 4.13, Path B)

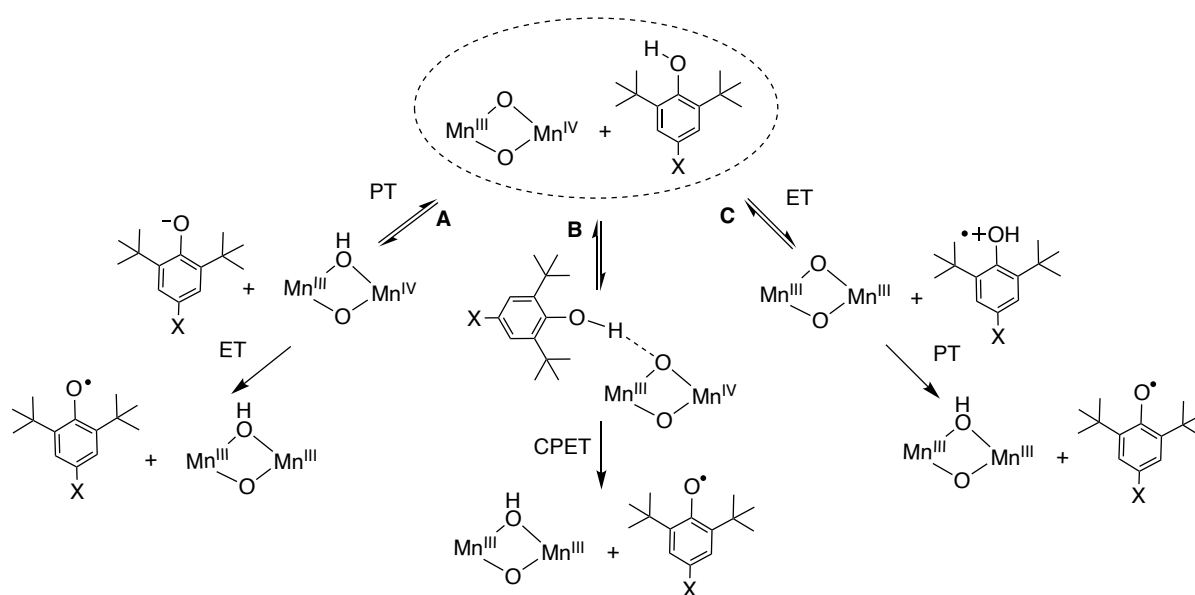


Figure 4.13: Three possible reactions mechanisms (A, B, or C) of **5** with 4-X-2,6-DTBP where $X = \text{OCH}_3$, CH_3 , $\text{C}(\text{CH}_3)_3$, H , C_2H_5 , and CN .

To gain a deeper understanding of the mechanism of the reaction between **5** and *para*-substituted phenols the kinetic parameters were calculated via construction of a Lineweaver-Burk plot for each reaction (A4.25 - A4.30).³¹ This was achieved upon linearisation of the plot of k_{obs} versus $[\text{S}]$ for each phenol according to the equation; $\frac{1}{k_{\text{obs}}} = \frac{K_M}{k_1} \frac{1}{[\text{S}]} + \frac{1}{k_1}$. This formula allowed for calculation of k_1 , the maximum rate of reaction, and K_M , the [substrate] at half the maximum rate for each phenol substrate (Table 4.2). Interestingly, it can be seen from table 4.2 that for the measured phenols as the O-H bond dissociation free energy (BDFE) increased the k_1 values decreased. This was easily

rationalised as the maximum rate of reaction (k_I) was reduced as the O-H bond of phenol became stronger and more difficult to break. In a similar analogy, the concentration of substrate required to reach half the maximum rate increased as the BDFE of the O-H bond of the phenol increased, meaning more substrate was required to drive the reaction as the bond became stronger.

Table 4.2: Kinetic parameters from the reaction of **5** with O-H bonds of varying strength.

Substrate	k_I (s ⁻¹)	K_M (M ⁻¹)	BDFE _{O-H} (kcal mol ⁻¹) ^a
[OH]-4-OCH ₃ -2,6-DTBP	0.086 (± 0.005)	0.002 (± 1x10 ⁻⁴)	73.8
[OD]-4-OCH ₃ -2,6-DTBP	0.032 (± 0.001)	0.0006 (± 1x10 ⁻⁵)	-
4-C ₂ H ₅ -2,6-DTBP	0.058 (± 0.001)	0.09 (± 3x10 ⁻³)	75.5
4-C(CH ₃) ₃ -2,6-DTBP	0.024 (± 0.001)	0.52 (± 0.01)	76.7
4-CH ₃ -2,6-DTBP	0.025 (± 0.001)	0.12 (± 0.01)	76.9
4-H-2,6-DTBP	0.023 (± 0.001)	0.02 (± 2x10 ⁻³)	78.3
4-CN-2,6-DTBP	0.014 (± 0.001)	0.81 (± 0.02)	79.4

^a = The BDFE_{O-H} values for the substrates were not available in a 1:9 MeCN/THF mixture hence we have reported BDFE_{O-H} values for benzene.

Using k_1 , the Gibbs energy of activation could be calculated to determine $\Delta G^\ddagger$ for each substrate. Plotting $\Delta G^\ddagger$ versus the BDFE_{O-H} associated with each phenol yielded a Bell-Evans-Polyani (BEP) plot with a slope of 0.15 (Figure 4.14).^{32,33} According to Marcus theory, the ideal BEP slope for a HAT is predicted to be 0.5.^{34,35} **5** falls on the edge of the range of slopes reported for systems ascribed to hydrogen atom transfer (0.15 - 0.7), indicating that HAT was the method **5** employed to oxidise phenols.^{34,36-44} A Marcus plot was constructed of $(RT/F)\ln(k_I)$ versus the substrates' redox potential (E_{ox}) to understand the rate-determining step. A linear fit of the Marcus plot afforded a slope of 0, however, it was worth noting that a weak correlation between the parameters was found with an R^2 value of just 0.56 (A4.43). A Marcus plot slope of -0.5 predicts a rate limiting ET process and a slope of -1.0 for rate limiting PT transfer.²⁸ A slope of 0 is associated with a rate limiting CPET mechanism. This further supported the postulate that **5** utilised a HAT mechanism during the oxidation of phenols. To understand the dependence of proton transfer during the rate determining step the correlation between the kinetic parameters and pK_a of the substrates was investigated. A plot of $\log(k_I)$ and $\log(K_M)$ versus the pK_a 's of the substrates revealed no relationship between these values (A4.45 and A4.46). This further indicated that PT was not involved in the rate-determining step and further affirmed a CPET during the oxidation of the phenol.⁴⁵

These results hinted that the source of this two-step mechanism for the reaction of **5** with the O-H bond substrates investigated was due to an initial association step of the phenol to the bis(μ -oxo) $Mn^{III}Mn^{IV}$ core followed by an irreversible one-electron oxidation via HAT (Path B, Figure 4.13). This association was essential to reactivity, as the proton of the phenol O-H bond coordinated to the bis(μ -oxo) $Mn^{III}Mn^{IV}$ core and acted as Lewis acid. This increased the electrophilic character of the core, therefore, activating it towards O-H bond oxidation of the phenol. The coordination of the phenol to the $Mn^{III}Mn^{IV}$ core was a

reversible process but the formation of the associated complex was required for the oxidation to occur. The oxidation of phenol to phenoxyl radical species by the activated $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species represented the second, irreversible step and rationalised the saturation kinetics observed for all phenols. Although rare, this association mechanism was not without precedence, Jackson and co-workers reported a $\text{Mn}^{\text{III}}\text{-OH}$ complex ($[\text{Mn}^{\text{III}}(\text{OH})(\text{dpaq})]^+$) that showed similar saturation behaviour in its reaction with *para*-substituted 2,6-di-*tert*-butylphenols.²⁹ Jackson *et al.* postulated that the $\text{Mn}^{\text{III}}\text{-OH}$ formed a hydrogen bonded adduct with the O-H bond of the phenol in the initial step followed by irreversible oxidation of the phenol.

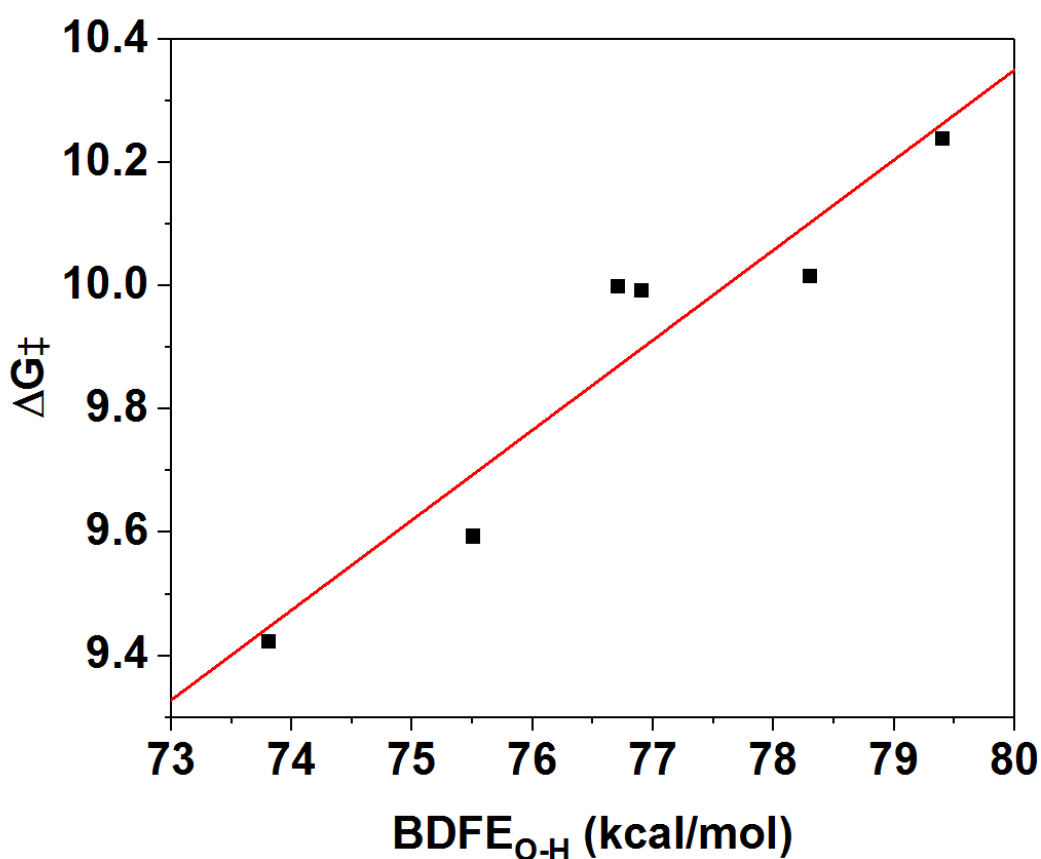


Figure 4.14: BEP plot of $\Delta G^\ddagger$ versus the $\text{BDFE}_{\text{O-H}}$ values for the substrates. Values of $\Delta G^\ddagger$ were calculated from first-order constants k_1 via the Eyring equation (slope = 0.15). The BDFE values for the substrates were not available in THF, hence, $\text{BDFE}_{\text{O-H}}$ values were plotted in benzene.

The kinetic isotope effect (KIE) was calculated using [OH]-4-OCH₃-2,6-DTBP and [OD]-4-OCH₃-2,6-DTBP substrates (A4.48 and A4.49). The deuterated phenol also displayed a Michaelis-Menten saturation type behaviour, indicating that deuteration did not affect the two-step mechanism. From this, a KIE value of 2.7 was determined for **5**. This falls into the classic KIE range (2-7), emblematic of a proton, or hydrogen atom transfer involved in the rate-limiting step. A KIE value >1 implied that O-H bond breakage occurred in the rate-determining step. Therefore, as the O-H bond must be intact in the rate-determining step and this further confirmed that the association mechanism of path B was employed during the oxidation of phenols. [Mn^{III}(OH)(dpaq)]⁺, a Mn complex that also undergoes an association mechanism with phenols displayed a KIE of 1.8 and also utilised a HAT mechanism.²⁹ This combination of support for the association of the phenol substrate to the bis(μ -oxo) core of **5** followed by irreversible HAT lead us to postulate that **5** followed pathway B during oxidation of O-H bonds (Figure 4.13).

The assignment of association mechanism followed by HAT for **5** was also consistent with our observations of linear kinetics from **4** in its reaction O-H bond containing substrates (Chapter 3). We postulated that the structure of **4** and **5** differ only by one proton that resided on the oxo diamond core of **4** and was absent in **5**. The absence of saturation kinetics in the reaction of **4** with substrates revealed that the association step was not required to activate **4** (Figure 4.15). We postulated that the presence of the proton in the core of **4** acted as an electron withdrawing species, removing electron density from the bis(μ -oxo) core, in turn increasing the electrophilicity of the core and therefore negated the association requirement.

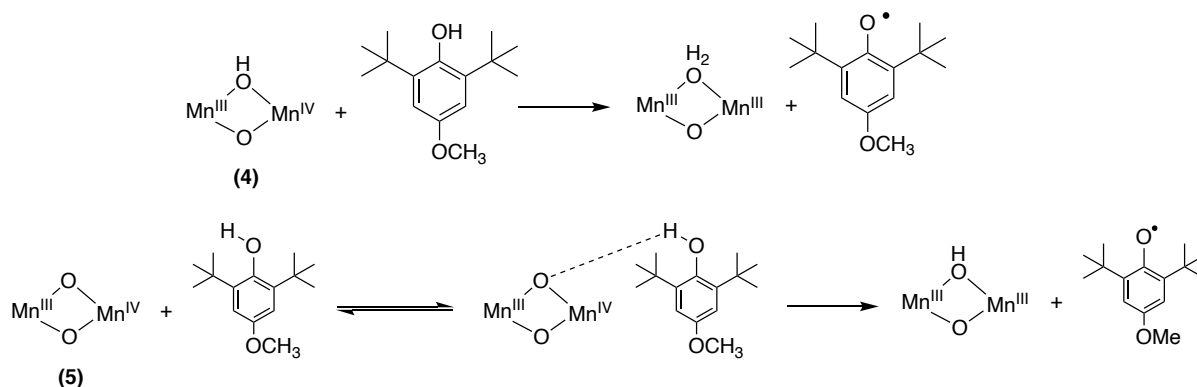


Figure 4.15: The mechanisms used by complexes **4** and **5** during the reaction with O-H bond containing substrates.

Interestingly, complexes **4** and **5** displayed also identical slopes of 0.16 and 0.15 respectively in their BEP plots (Table 4.3). This indicated that both complexes were utilising a HAT mechanism for the oxidation of O-H bonds. The similarity between the oxidation mechanism between the two complexes did not end there. The slopes of the plot of $\log(k_1)$ versus the bond dissociation energies (BDE) of the O-H bonds of substrates for complexes **4** and **5** were identical at -0.12, further indicating the likeness of the complexes (A3.44 and A4.50). The final similarity between complexes **4** and **5** was the KIE values of 2.5 and 2.7 respectively. The KIE values for both complexes fall within the classical range and indicated that proton or HAT was involved in the rate-limiting step. The number of parallels between the kinetic values of complexes **4** and **5** indicated that both complexes utilised a similar mechanism during oxidation. The similar kinetic values of **4** and **5** implied that both complexes utilised a similar species for oxidation. We postulated that following association of the phenol to complex **5** its' bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core resembles the (μ -hydroxo)(μ -oxo) structure of **4**, and resulted in similar kinetic values. Therefore, we reason that as complexes **4** and **5** displayed similar values this further validated the association mechanism of **5**.

Table 4.3: Slopes of the key kinetic graphs for complexes **4** and **5**.

	4	5
BEP slope	0.16 ($\pm$ 0.01)	0.15 ($\pm$ 0.02)
Log(k_1) vs BDE slope	-0.12 ($\pm$ 0.01)	-0.12 ($\pm$ 0.03)
KIE	2.5 ($\pm$ 0.01)	2.7 ($\pm$ 0.01)

The discovery that an unprotonated bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ utilised an association mechanism had implications for the catalytic cycle of Ib RNRs. We postulated that measurement of linear vs saturation kinetics of the oxidation of tyrosine residue in the enzyme would provide insight into the protonation state of the active $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. This would be of great interest as the protonation state of $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species has long been postulated but never confirmed experimentally.³

4.5 Conclusions

This, to our knowledge, is one of the first reported small molecule mimics of the Ib RNRs capable to completely reproduce the proposed catalytic cycle and reactivity found in the enzyme. Our work provides important mechanistic information such as providing further evidence that a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species is responsible for tyrosine oxidation. Analysis of the kinetics of **5**'s oxidation of phenols not only sheds light on the possible method of hydrogen atom transfer in class Ib RNRs but also justified the role of the proton for activating the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core towards phenol oxidation. This information could help in the future design of anti-cancer and anti-viral drugs.

4.6 Experimental

Materials

All reactions with air-sensitive materials were performed in a glove box in a N₂ atmosphere or under inert conditions using inert Schlenk line techniques. All reagents and solvents were purchased from commercial sources. Anhydrous *N,N*-dimethylformamide (DMF) was purchased and used without further purification. Anhydrous tetrahydrofuran (THF), acetonitrile (MeCN), and diethyl ether (Et₂O) were dispensed through an MBraun MB-SPS-5 Solvent Purification System and deoxygenated by purging with argon.

N,N-bis(2-picoyl)amine and 2,6-bis(chloromethyl)-4-methylphenol were synthesised as previously described.^{46,47} The ligand 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol (HBPMP) was synthesised as previously reported.⁴⁸ The synthesis of [Mn₂(O₂CCH₃)₂(BPMP)](ClO₄), **1**, was carried out as previously reported.⁴⁹

Caution: KO₂ and perchlorate salts of metal complexes are potentially explosive and must be handled with care and in small quantities. No problems were encountered during this project.

Physical Methods

¹H NMR analysis was performed on a Bruker Avance III 400 MHz instrument. Electrospray ionisation (ESI) mass spectra were obtained using micromass time of flight spectrometer (TOF), interfaced to a Waters 2690 HPLC, or by direct injection in the mass spectrometry instrument. Electronic absorption spectra were recorded in quartz cuvettes on a Hewlett Packard (Agilent) 8453 diode array spectrophotometer (190 – 1100 nm range) coupled to a liquid nitrogen cooled cryostat from Unisoku Scientific Instruments (Osaka, Japan). Electronic paramagnetic resonance (EPR) measurements were conducted on a Bruker Elexsys E-500 spectrometer with an Oxford ESR 910 liquid helium cryostat and an

Oxford temperature controller. The 30 K EPR quantification of the signals was relative to a $[\text{Cu}(\text{NO}_3)_2]$ spin standard in CH_3OH . The 77 K EPR quantification of the signals were relative to a $\text{TEMPO}\cdot$ spin standard in 1:9 MeCN/THF. EPR spectra of the phenoxyl radicals were recorded at 77 K, 9.2 GHz, 2.02 mW microwave power, with a 60 mT field sweep in 84 s, and 0.3 mT field modulation amplitude. Integration, simulation, and fitting were performed with Matlab and the easySpin computational package.⁵⁰ The simulation for the phenoxyl radical spectra was modelled as an $S = \frac{1}{2}$ electron spin with an isotropic g tensor. X-ray absorption spectroscopy methods: The Mn-K-edge X-ray absorption data were collected on beamline 7-3 of the Stanford Synchrotron Radiation Lightsource (SLAC National Accelerator Lab, Menlo Park, CA, USA). Data were collected with the storage ring operating at 3.0 GeV and 500 mA, using a LN2 cooled Si(220), $\phi = 90^\circ$ double-crystal monochromator, calibrated by using the first inflection point of a Mn foil (6539.0 eV). The monochromator was detuned by $\sim 50\%$ for higher harmonic rejection. A Canberra 32-element solid state germanium detector was used for fluorescence detection. All the measurements were performed at ambient pressure at ~ 17 K using an Oxford helium cryostat that was cooled by closed-cycle He gas loop. The parameters used for the scans were the following: 10 eV steps/1 second integration time in the pre-edge region, 0.3 eV steps /2 second integration time in the edge, and 0.05k steps in the EXAFS, with integration time increasing in a k^2 -weighted fashion from 2 to 4 seconds over the energy range ($k_{\text{max}} = 12.1\text{k}$). The total detector counts were typically 3-7k, well within the linear range of the detector electronics. Each sample was monitored for photoreduction. Complex **2** was found to be photoreduced by the X-rays and so only one scan was obtained per spot. Delrin ® Mossbauer/XAS cups with sample windows of 4 mm x 10 mm. Elaboration of the XAS data, including averaging, background removal and normalization, was performed using Athena.⁵¹ Edge energies were obtained by taking the first derivative of the rising edge,

while pre-edge energies were identified using the second derivative. Peak fitting analysis employed the program Fityk,⁵² using the protocol developed by Westre et al. for analysing Fe K-edge pre-edge peaks.⁵³ Briefly, a 15-18 eV segment of the normalized $\mu(E)$ spectrum was fit around the pre-edge, with pre-edge peaks modelled using pseudo-Voigt functions having a fixed 50:50 mixture of Gaussian: Lorentzian character, while the background absorption was modelled with a single pseudo-Voigt function where the proportion of Gaussian: Lorentzian character was allowed to float. Four slightly different energy ranges were fit separately, and the results averaged to generate the reported peak fits. Gas chromatography experiments have been performed using a ThermoFisher TRACE™ 1300 Gas Chromatograph equipped with a Flame Ionisation Detector. Hydrogen was provided by a Parker Hydrogen Gas Generator 20H-MD. Air was provided by Parker Zero Air Generator UHP-10ZA-S. The column used was a ThermoFisher TraceGOLD TG-1MS GC column

General Procedure for the reaction of 1 with cryptand

A 1:9 CH₃CN/THF solution (1.5 mM) of complex **1** was prepared. In a quartz cuvette 2 mL of a 1.5 mM solution of complex **1** was cooled to -90 °C. KO₂ (0.0056 g, 0.04 M) and cryptand (0.06 g, 0.08 M) were dissolved in DMF (2 mL). 75 μ L of this solution was added to the solution containing **1**. The reaction progress was monitored using electronic absorption spectroscopy. Lower yields were observed (less intense absorption features) with high equivalencies of KO₂ and at higher concentrations of **1**.

General Procedure for the generation of 3

A solution of **2** was prepared in a quartz cuvette at -90 °C as outlined in the procedure above. **5** was prepared by heating **2** from -90 °C to +20 °C over a fifteen minute period. The reaction progress was monitored using electronic absorption spectroscopy.

EPR Sample Preparation

Samples for EPR analysis were prepared by transferring ~ 1 mL of the desired solution from the quartz cuvette at -90 °C, via a pre-cooled pipette into a pre-cooled EPR tube and immediately freezing it in liquid nitrogen. The EPR spectra were recorded at 2 K (9.64 GHz, 0.2 mW microwave power).

Reactivity Studies

A solution of **5** in 1:9 CH₃CN/THF at -90 °C was prepared using the above procedure. Substrates were added as concentrated 1:9 CH₃CN/THF solutions to solutions of **5** at +20 °C. The reactions were monitored using electronic absorption spectroscopy.

Preparation of K¹⁸O₂:

Method was adapted from a similar procedure reported by Tolman *et al.*⁵⁴ TEMPO-H (0.159 g, 1 mmol) was reacted with potassium hydride (KH) in 2 ml of THF. Solution was allowed to stir for two hours until gas evolution ceased. Solution was filtered through a 0.2 μm syringe filter and the filter washed with an addition 2 ml THF. Solution freeze pump thawed to ensure an environment free of ¹⁶O₂. The solution was frozen in liquid nitrogen and the headspace evacuated and refilled with ¹⁸O₂. Solution was allowed to warm to room temperature and upon this changed from a colourless solution to a dark orange colour. The solvent was removed under vacuum resulting in a dark orange residue. The residue was washed with dry, degassed hexane to yield a yellow precipitate. The hexane was decanted

and the powder was washed several times with hexane until the decanted liquid was colourless and no longer orange. The yellow powder was then dried under vacuum and used without further purification.

Synthesis of 4-methoxy-2,6-di-tert-butylphenoxy radical

4-methoxy-2,6-di-tert-butylphenoxy radical was synthesised according to a previously reported procedure.⁵⁵ The purple oil obtained was washed three times with deoxygenated diethyl ether (Et₂O), the solvent removed and the oil dried under vacuum for one hour. It was then stored in a nitrogen atmosphere glovebox where the solutions for electronic absorption spectroscopy were prepared in deoxygenated 1:9 MeCN/THF.

4.7 References

- (1) Cotruvo, J. A.; Stubbe, J. *Annu. Rev. Biochem.* **2011**, *80* (1), 733–767.
- (2) Cotruvo, J. A.; Stubbe, J. *Biochemistry* **2010**, *49* (6), 1297–1309.
- (3) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, *135* (10), 4027–4039.
- (4) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, *57* (4), 918–922.
- (5) Magherusan, A. M.; Kal, S.; Nelis, D. N.; Doyle, L. M.; Farquhar, E. R.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2019**, *58* (17), 5718–5722.
- (6) Leto, D. F.; Chattopadhyay, S.; Day, V. W.; Jackson, T. A. *Dalton. Trans.* **2013**, *42* (36), 13014–13025.
- (7) Groni, S.; Dorlet, P.; Blain, G.; Bourcier, S.; Guillot, R.; Anxolabéhère-Mallart, E. *Inorg. Chem.* **2008**, *47* (8), 3166–3172.
- (8) Brewer, K. J.; Calvin, M.; Lumpkin, R. S.; Otvos, J. W.; Spree, L. O. *Inorg. Chem.* **1989**, *28* (25), 4446–4451.
- (9) Dubois, L.; Xiang, D. F.; Tan, X. S.; Pécaut, J.; Jones, P.; Baudron, S.; Le Pape, L.; Latour, J. M.; Baffert, C.; Chardon-Noblat, S.; Collomb, M. N.; Deronzier, A. *Inorg. Chem.* **2003**, *42* (3), 750–760.
- (10) Gelasco, A.; Kirk, M. L.; Kampf, J. W.; Pecoraro, V. L. *Inorg. Chem.* **1997**, *36* (9), 1829–1837.
- (11) Rose, H. R.; Ghosh, M. K.; Maggiolo, A. O.; Pollock, C. J.; Blaesi, E. J.; Hajj, V.; Wei, Y.; Rajakovich, L. J.; Chang, W. C.; Han, Y.; Hajj, M.; Krebs, C.; Silakov, A.; Pandelia, M. E.; Bollinger, J. M.; Boal, A. K. *Biochemistry* **2018**, *57* (18), 2679–2693.
- (12) Teutloff, C.; Schäfer, K. O.; Sinnecker, S.; Barynin, V.; Bittl, R.; Wieghardt, K.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **2005**, *43* (SPEC. ISS.), S51–S64.
- (13) Goodson, P. A.; Hodgson, D. J.; Glerup, J.; Michelsen, K.; Pedersen, E. *Inorg.*

- Chem.* **1990**, 29 (3), 503–508.
- (14) Messinger, J.; Nugent, J. H. A.; Evans, M. C. W. *Biochemistry* **1997**, 36 (37), 11055–11060.
 - (15) Teutloff, C.; Schäfer, K. O.; Sinnecker, S.; Barynin, V.; Bittl, R.; Wieghardt, K.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **2005**, 43 (SPEC. ISS.), 51–64.
 - (16) Dismukes, G. C.; Sheats, J. E.; Smeeal, J. A. *J. Am. Chem. Soc.* **1987**, 109 (23), 7202–7203.
 - (17) Mahapatra, S.; Das, P.; Mukherjee, R. *Dalton. Trans.* **1993**, No. 2, 217–220.
 - (18) Howard, T.; Telser, J.; DeRose, V. J. *Inorg. Chem.* **2000**, 39 (15), 3379–3385.
 - (19) Golombek, A. P.; Hendrich, M. P. *J. Magn. Reson.* **2003**, 165 (1), 33–48.
 - (20) Schreiber, R. E.; Cohen, H.; Leitus, G.; Wolf, S. G.; Zhou, A.; Que, L.; Neumann, R. *J. Am. Chem. Soc.* **2015**, 137 (27), 8738–8748.
 - (21) Stemmler, T. L.; Sossong, T. M.; Goldstein, J. I.; Ash, D. E.; Elgren, T. E.; Kurtz, D. M.; Penner-Hahn, J. E. *Biochemistry* **1997**, 36 (32), 9847–9858.
 - (22) Celorrio, V.; Leach, A. S.; Huang, H.; Hayama, S.; Freeman, A.; Inwood, D. W.; Fermin, D. J.; Russell, A. E. *ACS Catal.* **2021**, 11, 6431–6439.
 - (23) Yano, J.; Kern, J.; Irrgang, K. D.; Latimer, M. J.; Bergmann, U.; Glatzel, P.; Pushkar, Y.; Biesiadka, J.; Loll, B.; Sauer, K.; Messinger, J.; Zouni, A.; Yachandra, V. K. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, 102 (34), 12047–12052.
 - (24) Spedalotto, G.; Lovisari, M.; McDonald, A. R. *ACS Omega* **2021**, 6 (42), 28162–28170.
 - (25) Dixon, M.; Webb, E. C. *Enzymes*, 3rd ed.; Longman Group Ltd: Harlow, UK, 1979.
 - (26) Johnson, K. A.; Goody, R. S. *Biochemistry* **2011**, 50 (39), 8264–8269.
 - (27) Kasai, N.; Ikushiro, S.; Hirose, S.; Arisawa, A.; Ichinose, H.; Uchida, Y.; Wariishi, H.; Ohta, M.; Sakaki, T. *J. Biochem.* **2010**, 147 (1), 117–125.
 - (28) Mondal, P.; McDonald, A. R. *Chem. – A Eur. J.* **2020**, 26 (44), 10083–10089.
 - (29) Wijeratne, G. B.; Corzine, B.; Day, V. W.; Jackson, T. A. *Inorg. Chem.* **2014**, 53 (14), 7622–7634.
 - (30) Layfield, J. P.; Hammes-Schiffer, S. *Chem. Rev.* **2014**, 114 (7), 3466–3494.
 - (31) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, 56 (3), 658–666.
 - (32) Onald, R.; Bell, P. *Proc. R. Soc. London. Ser. A - Math. Phys. Sci.* **1936**, 154 (882), 414–429.
 - (33) Evans, M. G.; Polanyi, M. *Trans. Faraday Soc.* **1937**, 33, 448–452.
 - (34) Roth, J. P.; Lovell, S.; Mayer, J. M. *J. Am. Chem. Soc.* **2000**, 122 (23), 5486–5498.
 - (35) Mayer, J. M. *Acc. Chem. Res.* **2010**, 44 (1), 36–46.
 - (36) Mondal, P.; Pirovano, P.; Das, A.; Farquhar, E. R.; McDonald, A. R. *J. Am. Chem. Soc.* **2018**, 140 (5), 1834–1841.
 - (37) Mayer, J. M. *Acc. Chem. Res.* **2011**, 44 (1), 36–46.
 - (38) Gardner, K. A.; Kuehnert, L. L.; Mayer, J. M. *Inorg. Chem.* **1997**, 36 (10), 2069–2078.
 - (39) Goldsmith, C. R.; Jonas, R. T.; Stack, T. D. P. *J. Am. Chem. Soc.* **2002**, 124 (1), 83–96.
 - (40) Bryant, J. R.; Mayer, J. M. *J. Am. Chem. Soc.* **2003**, 125 (34), 10351–10361.
 - (41) Yin, G.; Danby, A. M.; Kitko, D.; Carter, J. D.; Scheper, W. M.; Busch, D. H. *J. Am. Chem. Soc.* **2008**, 130 (48), 16245–16253.
 - (42) Wang, D.; Zhang, M.; Bühlmann, P.; Que, L. *J. Am. Chem. Soc.* **2010**, 132 (22), 7638–7644.
 - (43) Dhar, D.; Tolman, W. B. *J. Am. Chem. Soc.* **2015**, 137 (3), 1322–1329.
 - (44) Mandal, D.; Shaik, S. *J. Am. Chem. Soc.* **2016**, 138 (7), 2094–2097.

- (45) Goetz, M. K.; Anderson, J. S. *J. Am. Chem. Soc.* **2019**, *141* (9), 4051–4062.
- (46) Borovik, A. S.; Que, L.; Papaefthymiou, V.; Taylor, L. F.; Anderson, O. P. *J. Am. Chem. Soc.* **1989**, *111* (16), 6183–6195.
- (47) Raycroft, M. A. R.; Maxwell, C. I.; Oldham, R. A. A.; Andrea, A. S.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2012**, *51* (19), 10325–10333.
- (48) Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, *39* (16), 3526–3536.
- (49) Suzuki, M.; Mikuriya, M.; Murata, S.; Uehara, A.; Oshio, H.; Kida, S.; Saito, K. *Bull. Chem. Soc. Jpn.* **1987**, *60* (12), 4305–4312.
- (50) Stoll, S.; Schweiger, A. *J. Magn. Reson.* **2006**, *178* (1), 42–55.
- (51) Ravel, B.; Newville, M. *J. Synchrotron Radiat.* **2005**, *12* (4), 537–541.
- (52) Wojdyr, M. *J. Appl. Crystallogr.* **2010**, *43* (5), 1126–1128.
- (53) Westre, T. E.; Kennepohl, P.; DeWitt, J. G.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1997**, *119* (27), 6297–6314.
- (54) Donoghue, P. J.; Gupta, A. K.; Boyce, D. W.; Cramer, C. J.; Tolman, W. B. *J. Am. Chem. Soc.* **2010**, *132* (45), 15869–15871.
- (55) Wittman, J. M.; Hayoun, R.; Kaminsky, W.; Coggins, M. K.; Mayer, J. M. *J. Am. Chem. Soc.* **2013**, *135* (35), 12956–12959.

Chapter 5: Lewis Acid Activation of a Mn^{II}Mn^{III}-Peroxide with Relevance to the Catalytic Cycle of Ib RNRs.

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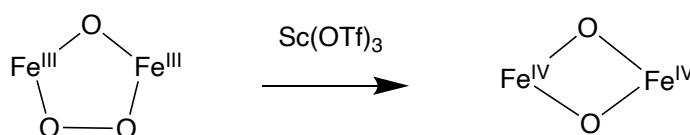
5.0 Abstract

Addition of 1 eq. of $\text{Sc}(\text{OTf})_3$ to a 1.5 mM solution of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**) at $-90\text{ }^{\circ}\text{C}$ resulted in the formation of a new species (**6**) as evidenced by the new UV-vis features at $\lambda_{\text{max}} = 410$ and 550 nm . Analysis of this new species **6** was performed using electron paramagnetic spectroscopy, X-ray absorption near edge spectroscopy, and mass spectrometry. New species **6** was found to be a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex with a possible Sc^{3+} ion associated to the diamond core. The reactivity of this new complex was investigated and **6** was determined to be capable of the one-electron oxidation of O-H bond-containing substrates. Further kinetic analysis was performed on the reaction of **6** with 4-OCH₃-TEMPO-H and revealed that **6** reacted via a linear, single step reaction with a second-order rate constant of $k_2 = 0.96\text{ M}^{-1}\text{ s}^{-1}$ ($\pm 0.03\text{ M}^{-1}\text{ s}^{-1}$). **6** was found to react approximately four times faster than the similar $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (**4**) with the same O-H bond-containing substrate. We postulated the increase in rate was due to the association of the Sc^{3+} ion to the $\text{bis}(\mu\text{-oxo})$ core of **6**. This association increased the electrophilicity of the core and resulted in the increased rate of reaction.

5.1 Introduction

During the catalytic cycle of Ib RNRs, it was postulated that a proton was involved in the conversion of the postulated $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species to the $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species that was responsible for the oxidation of tyrosine.¹ We have demonstrated that the addition of a proton to our $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide mimic (**2**) can initiate the conversion to a $(\mu\text{-oxo})(\mu\text{-hydroxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (**4**) capable of the one-electron oxidation of O-H bond-containing substrates (Chapter 3). Recently, the Que group has demonstrated that the addition of the Lewis acid Sc^{3+} promoted O-O bond cleavage in a $(\mu\text{-1,2-peroxo})\text{Fe}^{\text{III}}_2$ complex to generate a $\text{bis}(\mu\text{-oxo})\text{Fe}^{\text{IV}}_2$ core (Figure 5.1).² Therefore, we were interested in exploring if addition of Sc^{3+} to our related $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complex (**2**) would also result in O-O bond cleavage and generation of a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species.

Que 2020:



This Work:

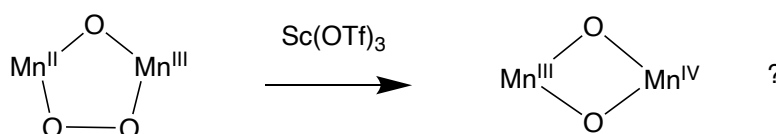


Figure 5.1: Top: Reactivity observed by Que upon addition of $\text{Sc}(\text{OTf})_3$ to a $(\mu\text{-1,2-peroxo})\text{Fe}^{\text{III}}_2$ complex.² Bottom: The reactivity to be explored in this work.

5.2 Results and Discussion

To a 1.5 mM solution of **2** in 1:9 MeCN/THF at -90 °C, 1 eq. of scandium trifluoromethanesulfonate ($\text{Sc}(\text{OTf})_3$) was added and the reaction was monitored via UV-vis spectroscopy (Figure 5.2). An immediate reaction was observed, evidenced by the change in electronic absorption features of **2** and formation of new features associated with the generation of a new species (**6**). New species **6** displayed two new bands in the UV-vis spectrum with $\lambda_{\text{max}} = 410$ and 550 nm. Conversion of **2** to **6** completed in 150 seconds (A5.1). Sub-stoichiometric additions of $\text{Sc}(\text{OTf})_3$ to **2** resulted in lower yields of **6** as evidenced by lower intensity electronic absorption features (A5.2). Addition of $\text{Sc}(\text{OTf})_3 > 1$ eq. to **2** afforded no increase in the yields of **6** compared to when just 1 eq. was used (A5.3). Hence, 1 eq. of $\text{Sc}(\text{OTf})_3$ was chosen to generate **6** from **2**. Complex **6** was found to be metastable at -90 °C with a half-life of $t_{1/2} = 286$ hours.

The electronic absorption features displayed by **6** were characteristic of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes which typically display red-shifted UV-vis features in comparison to $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes. In fact, **6** shares similar UV-vis features to the $[\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}\text{L}_2]^{3+}$ complex reported by Girerd *et al.* which displayed a broad, intense band centred at $\lambda_{\text{max}} = 570$ nm.³ Girerd assigned the source of the feature to be a phenolate to Mn^{IV} charge transfer. This was analogous to complex **6** which is also supported by a phenolate containing ligand. Therefore, we postulated that the band at $\lambda_{\text{max}} = 550$ nm in the UV-vis spectrum of **6** originated from the phenolate to Mn^{IV} charge transfer in a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex. Moreover, the electronic absorption features of new complex **6** were similar to those of the (μ -oxo)(μ -hydroxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex (**4**, Chapter 3) analysed previously. Both complexes **6** and **4** presented an intense, broad absorption feature $\lambda_{\text{max}} = 550$ nm that trailed into the near-infrared region of the spectrum. Interestingly, the features

of new complex **6** appeared sharper and more defined in comparison to that of complex **4** (A5.4).

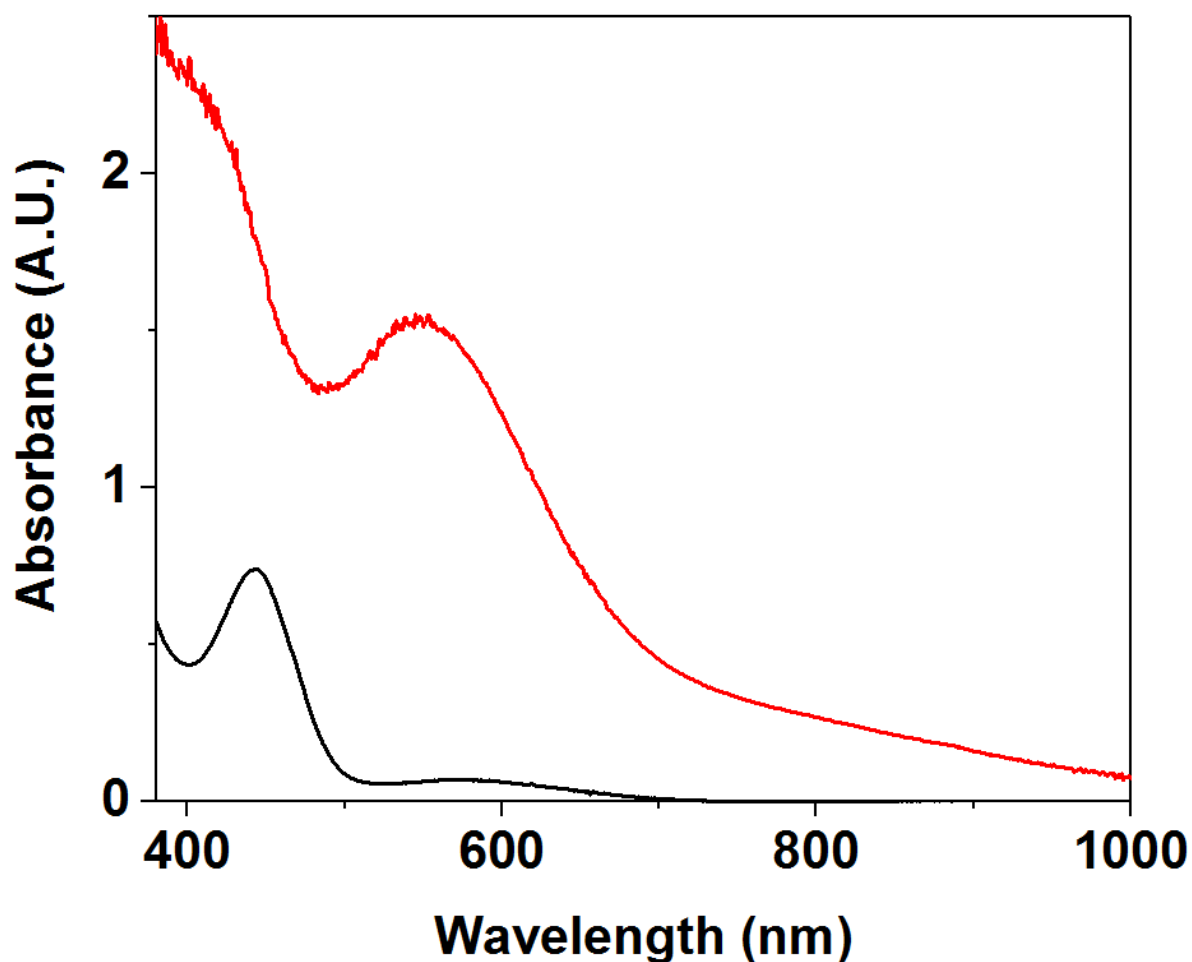


Figure 5.2: UV-vis spectra of the reaction of **2** (black trace) + 1 eq. $\text{Sc}(\text{OTf})_3$ (red trace) in 1:9 MeCN/THF at -90°C .

To further analyse the structure of new complex **6**, electronic paramagnetic resonance (EPR) spectroscopy was performed. At 10 K the EPR spectrum of **6** revealed two features; an 11-line signal centred at $g = 2.6$ and a 16-line signal centred at $g = 2$ (Figure 5.3). The EPR spectrum of **6** was also measured at 20 and 30 K. The two signals remained the same and no new features were observed at all three temperatures (Figure 5.3).

An 11-line signal indicated that two equivalent Mn centres were present in the sample $\{(2nI + 1) = (2 \times 2 \times 5/2 + 1) = 11\}$, where n = number of atoms, and $I = \text{Mn}^{55}$ nuclear spin = $5/2$.

For example, the Mn^{II}_2 complexes $[\text{Mn}^{\text{II}}_2(\text{TMEDA})_2(\text{OCCH}_3)_4(\text{H}_2\text{O})]$ and $[\text{Mn}^{\text{II}}_2(\text{Me}_3\text{TACN})_2(\mu\text{-OCCH}_3)_3]\text{BPh}_4$ both displayed 11-line signals centred at $g = 2.34$ and $g = 1.96$ respectively.^{4,5} Moreover, the 11-line signal observed in the spectra of **6** had an average hyperfine coupling constant (A_{av}) of 4.4 mT. This was consistent with previously characterised dinuclear Mn^{II} complexes which typically displayed A_{av} between 4.4 – 4.6 mT.^{4,5} We therefore assigned the 11-line signal to originate from a Mn^{II}_2 complex. The 11-line signal at $g = 2.6$ has been observed before in the EPR spectra of bis(μ -oxo) complexes **4** and **5** (Chapters 3 and 4 respectively). Initially we suspected that this signal belonged to unreacted Mn^{II}_2 starting material but this signal was not visible during variable EPR measurements of $[\text{Mn}^{\text{II}}_2(\text{OAc})_2(\text{BPMP})](\text{ClO}_4)$ (**1**).⁶ However, in the variable temperature EPR measurements of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide precursor **2** this 11-line was seen at $g = 2.6$ at 30 K (A3.12). Therefore, we postulated that the 11-line signal originated from a Mn^{II}_2 side product formed during the reaction of the $[\text{Mn}^{\text{II}}(\text{OAc})_2(\text{BPMP})](\text{ClO}_4)$ (**1**) with KO_2 .

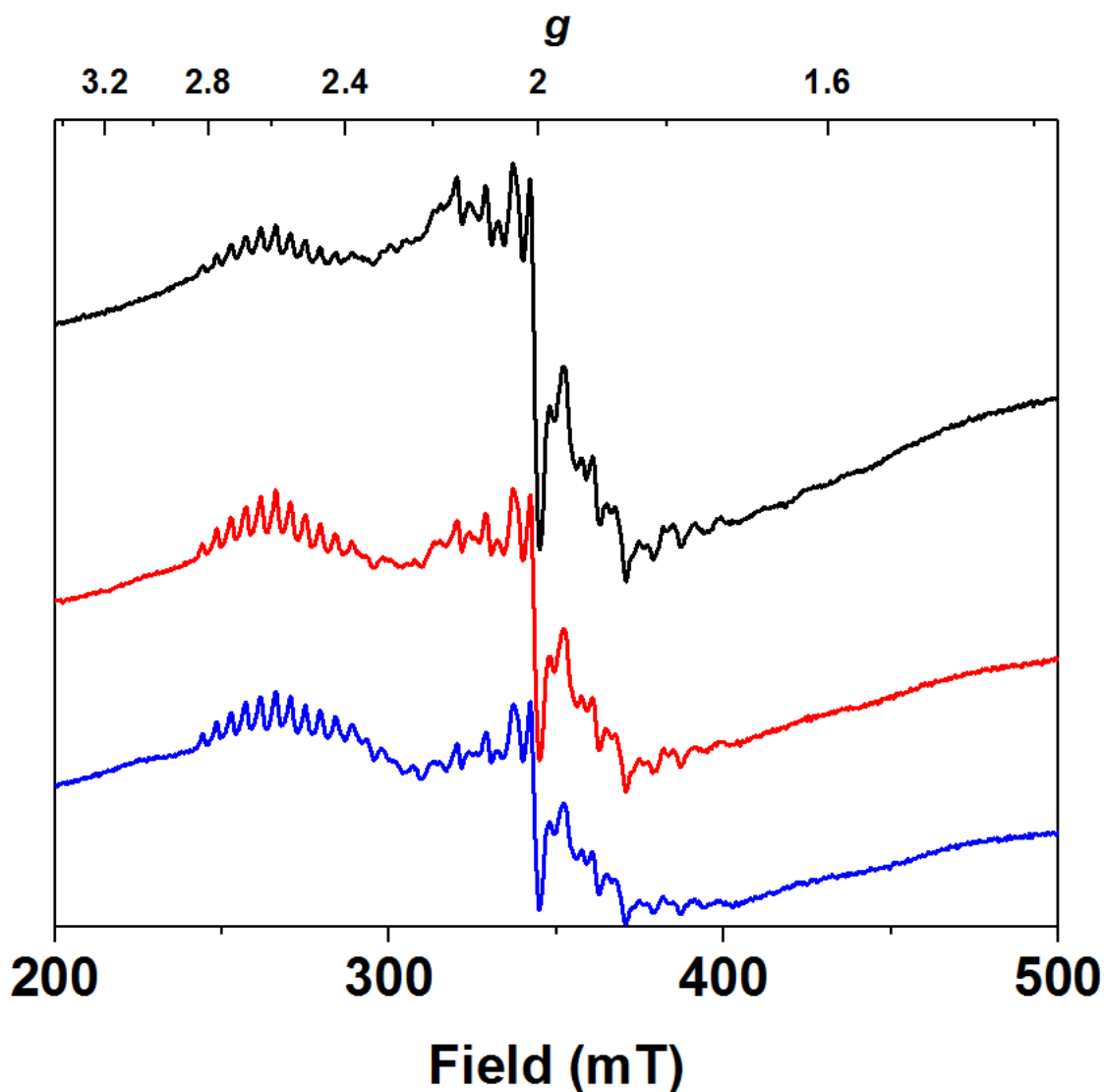


Figure 5.3: Perpendicular X-Band EPR spectra of **6** (generated from 1.5 mM **1** + 1 eq. KO_2 + 1 eq. $\text{Sc}(\text{OTf})_3$) measured at 10 K (black trace), 20 K (red trace), and 30 K (blue trace) (9.65 GHz, 0.2 mW microwave power).

The other signal observed in the EPR spectra of **6** at 10, 20, and 30 K was a 16-line signal centred at $g = 2$. This signal had a spectral width of 110 mT and an $A_{av} = 7.8$ mT. The number of lines, spectral width, and A_{av} was characteristic of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex.^{7–12} Moreover, the spectral features of the 16-line signal were still resolved at $g = 2$ at 30 K and further confirmed that the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** was converted to a new species **6**, as $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species are only resolved at temperatures < 10 K.¹³ In contrast, bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes do not suffer from this temperature sensitivity and their 16-line

signals can be clearly seen at temperatures up to 140 K.⁷ Therefore, we postulated that **6** was a new bis(μ -oxo)Mn^{III}Mn^{IV} species formed following addition of Sc³⁺ to **2**.

Interestingly, the EPR spectrum obtained for **6** was similar to that observed for the previously characterised bis(μ -oxo) complexes **4** and **5** (Chapters 3 and 4 respectively, Figure 5.4), with all three sharing similar number of lines, shape, spectral width and A_{av} values. One difference between the EPR spectra of new species **6** compared to previously characterised **4** and **5**, was the presence of what appeared to be an intense isotropic signal centred at $g = 2$ in the spectrum of **6**. It was unclear if this was a separate signal, or part of the 16-line bis(μ -oxo) signal. Organic radical species display isotropic signals centred at $g = 2$, however, their intensity is typically much greater compared to metal based signals.¹⁴ Due to the relatively low intensity of this signal we postulated that it is not an organic based signal and instead an artifact of the 16-line $g = 2$ signal. Due to the similarity of the EPR spectra reported by **4**, **5**, and **6** it appeared that all three complexes may have formed similar bis(μ -oxo)Mn^{III}Mn^{IV} structures. This indicated that the formation of bis(μ -oxo)Mn^{III}Mn^{IV} complexes are thermodynamically favoured following activation of Mn^{II}Mn^{III}-peroxides.

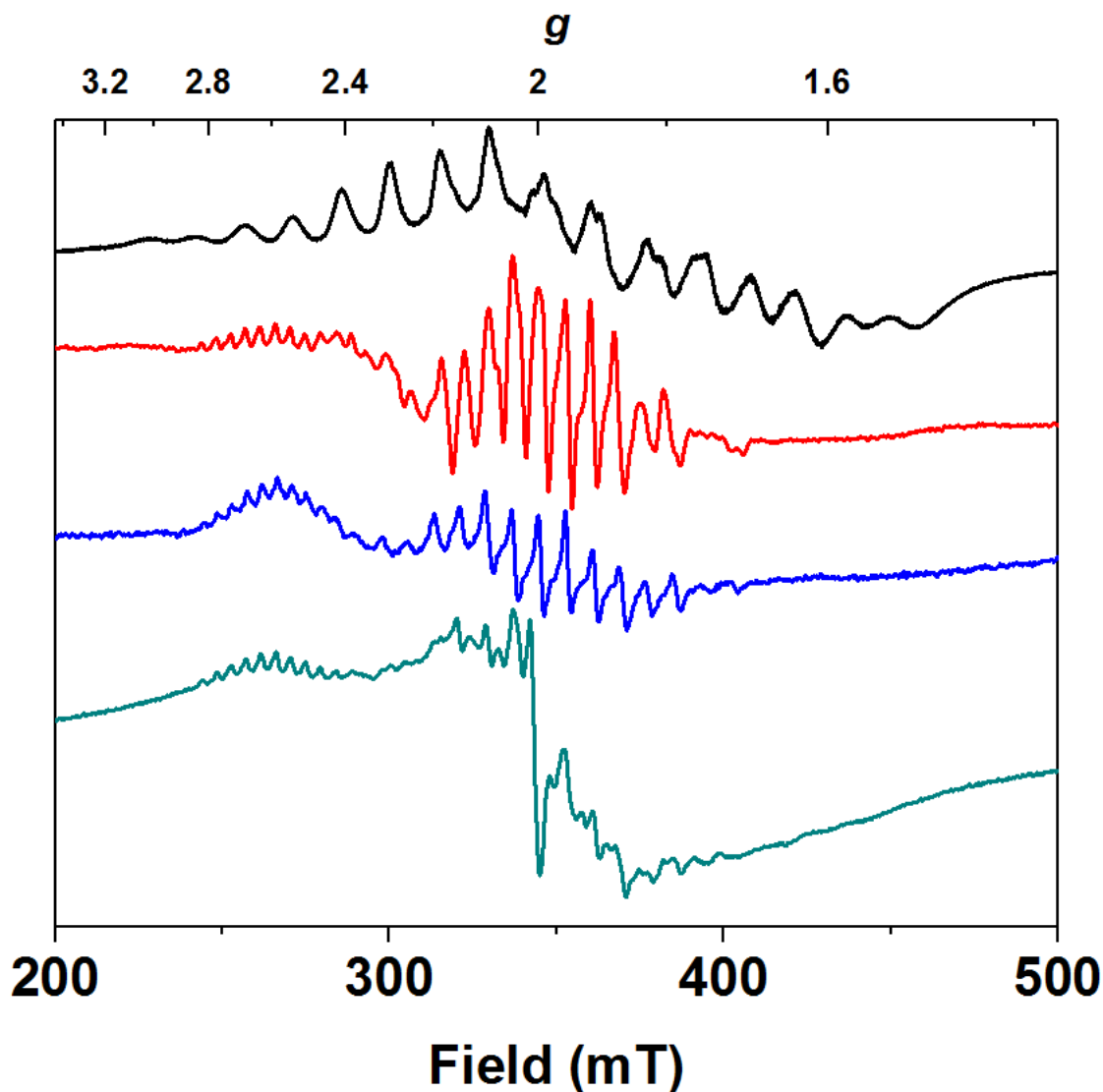


Figure 5.4: EPR spectra of **2** (black trace) recorded at 2 K, **4** (red trace) recorded at 7 K, **5** (blue trace) recorded at 30 K, and **6** (green trace) recorded at 11 K (9.65 GHz, 0.2 mW microwave power).

Cold-injection electrospray ionisation mass spectrometry (ESI-MS) of a just thawed methanol solution of **6** revealed a peak at $m/z = 335.5686$ which agrees well with the predicted peak of $m/z = 335.5682$ for the dication species $[\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}\text{BPMP}]^{2+}$ (Figure 5.5, left). The spacing between the peaks was just 0.5 units, which further confirmed the assignment of a dication species. Upon preparation of **6** using labelled K^{18}O_2 the peaks of the dication shifted by 2 mass units (Figure 5.5, right) consistent with two $^{18}\text{O}_2$ atoms incorporated into the dication. The peak at $m/z = 770.29$ associated with **2** was also

not present in the mass spectrum of **6** -further indicating that **2** was converted to **6** upon addition of $\text{Sc}(\text{OTf})_3$ (A5.6). The mass spectrometry data further confirmed what was indicated via EPR - that a $[\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}\text{BPMP}]^{2+}$ complex **6** was formed in the reaction of **2** + 1 eq. $\text{Sc}(\text{OTf})_3$.

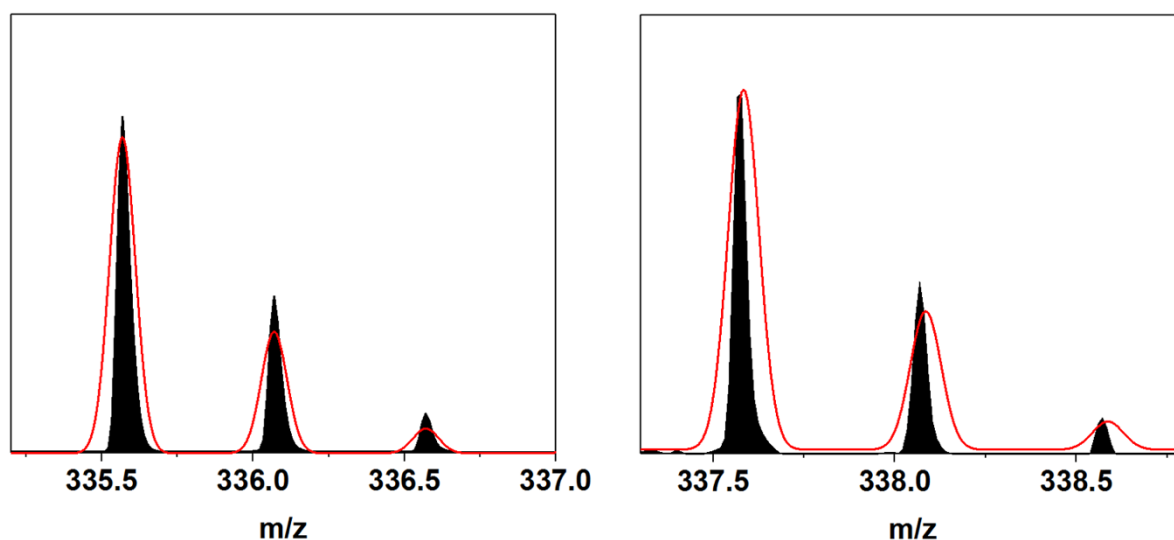


Figure 5.5: Left; ESI-MS spectra of **6** prepared using K^{16}O_2 (black trace) with simulated spectra (red trace). Right; ESI-MS spectra of **6** prepared using K^{18}O_2 (black trace) with simulated isotopic spectra (red trace).

X-ray diffraction quality crystals could not be obtained for complex **6** due to its thermal instability. Therefore, in order to further investigate the Mn oxidation states and their local geometries of **6**, Mn K-edge X-ray near edge spectroscopy (XANES) was employed. The XANES spectra of complex **6** revealed an edge energy of 6549.2 eV (Figure 5.6). The pre-edge area was modelled using a 50:50 pseudo-Voigt function (A5.8). The pre-edge centre and area were calculated to be 6540.7 eV and 14.5 units respectively.

Upon conversion from the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide precursor (**2**) to new complex **6** there was a Mn K-edge energy shift of 0.3 eV (Table 5.1). Previous XANES studies of Mn complexes have shown that an edge energy shift of between 2 – 4 eV was expected for every one unit increase of the formal oxidation state of the Mn centre.^{15,16} Therefore, an edge energy shift

of just 0.3 eV was much lower than what we expected for the postulated $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex. However, XANES spectroscopy is a measurement of all the Mn centres in the reaction mixture, and as revealed by EPR spectroscopy, there appeared to be some Mn^{II}_2 side product formed during the reaction. This could have the effect of “averaging down” the edge energy shift leading to the lower than expected value we observed.

Upon conversion from the low-valent Mn^{II}_2 starting material (**1**, black trace, Figure 5.6) to $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** (red trace, Figure 5.6) to **6** (blue trace, Figure 5.6), there was a gradual removal of the double “hump” feature at ~6552 eV that was completely absent in **6**. This double hump feature has been observed previously in the XANES spectrum of dinuclear manganese enzymes and was indicative of the presence of the Mn^{II}_2 .¹⁷ This showed that moving from **1** to **2** to **6** there was less and less Mn^{II}_2 material in each sample and indicated that a reaction of the Mn^{II}_2 had occurred and that less Mn^{II}_2 was present in the **6** compared to the precursors.

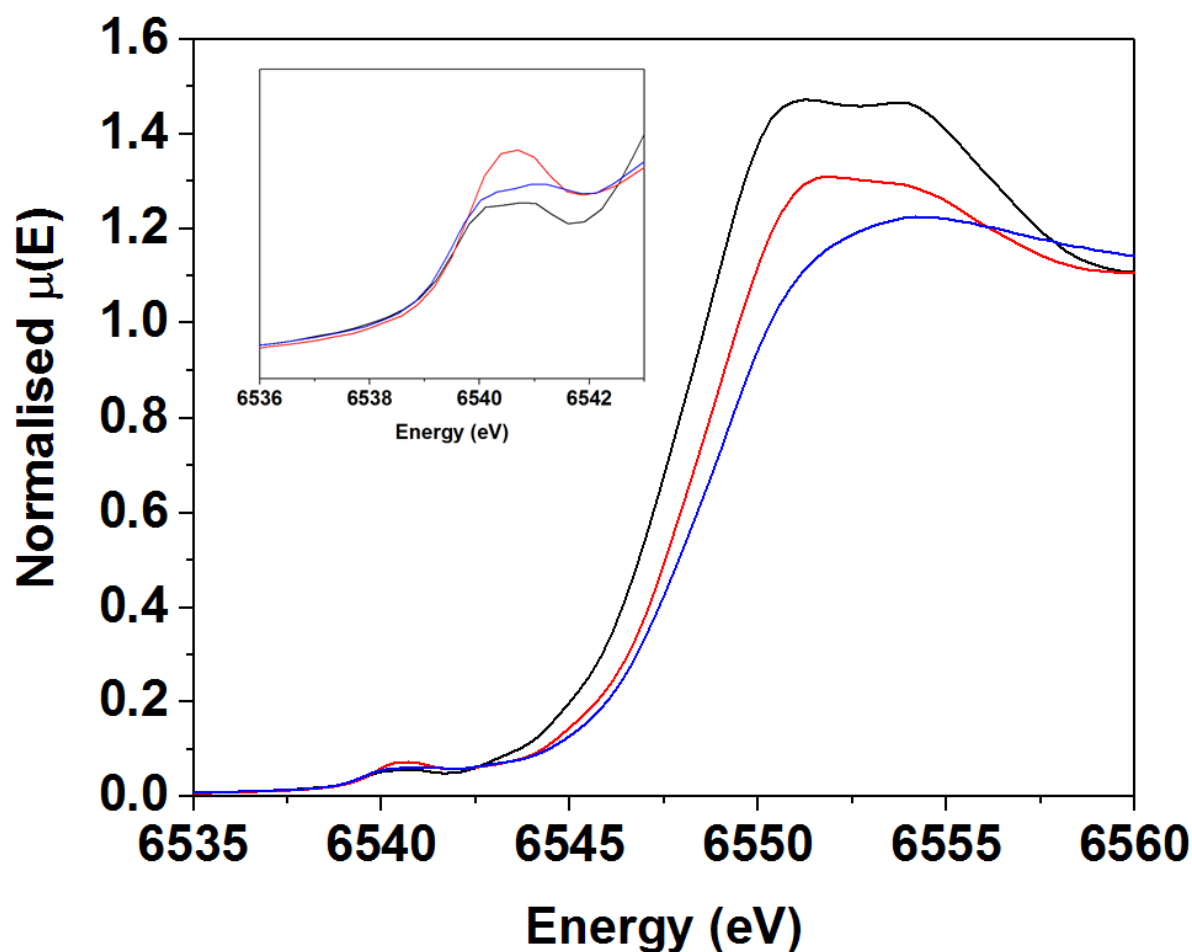


Figure 5.6: Normalised XANES spectra of **1** (black trace), **2** (red trace), and **6** (blue trace). Inset: Expansion of the pre-edge region.

The pre-edge peak was associated with crystal-field transitions from the 1s to 3d levels and therefore offered important information about the local geometry and structure of the metal centres.¹⁸ Modelling of the pre-edge peak before the main edge allowed for calculation of the area to be 14.5 units. This value of the pre-edge area was similar to the value recorded for the bis(μ -oxo)Mn^{III}Mn^{IV} species observed in the catalytic cycle of manganese catalase (14.5 ± 0.4 eV).¹⁸ This further indicated **6** to be a bis(μ -oxo)Mn^{III}Mn^{IV} complex. Upon conversion of **2** to **6** there was an increase in the edge area from 13.5 units to 14.5 units. This represented an overall increase of the area by one unit upon conversion from **2** to **6** (Table 5.1). An increase in the pre-edge area was associated with a decrease in the electronic symmetry around the metal centres.¹⁵ This was, once again, counter intuitive to

what we anticipated from the proposed bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex. In a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex each metal centre would be hexacoordinate and inherently more centrosymmetric than the pentacoordinate geometry of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, **2**. We postulated that the source of the increase in area was due to the association of the Sc^{3+} ion to the bis(μ -oxo) core which lead to a decrease in the electronic symmetry of the complex. It is possible that the presence of the triflate anion (OTf) in solution was instead the source of the breaking of symmetry in the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ rather than the Sc^{3+} ion, however, we postulated a similar association occurred in the case of complex **4** (Chapter 3), where a H^+ ion was associated with the bis(μ -oxo) core of the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species.

Complex **6** displayed a very similar edge energy compared to the previously characterised complexes **4** and **5**, indicating that all of these bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes appeared to suffer from the same lower edge energy shifts (Figure 5.7, Table 5.1). This appeared to be inherent to all of these complexes as they all showed the presence of the Mn^{II}_2 side product in their EPR spectra, which could be the source of the lower edge energies observed for all three complexes. Interestingly, **6** displayed a much higher pre-edge area compared to **4** and **5**. This supported the postulate that the associated Sc^{3+} introduced a lowering of the electronic symmetry of the bis(μ -oxo) core of **6** and resulted in the larger pre-edge area, as the cores of **4** and **5** did not experience this asymmetry.

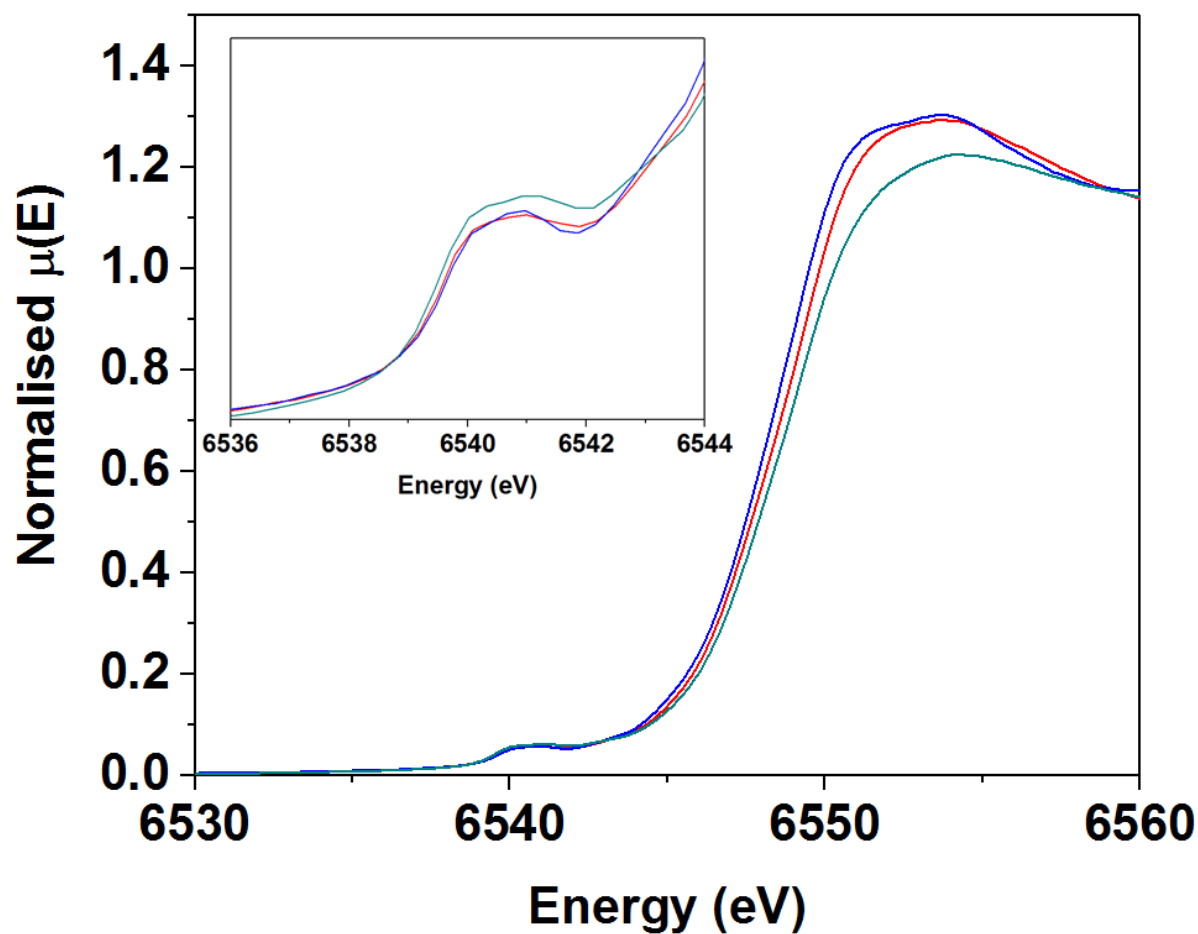


Figure 5.7: Normalised XANES spectra of **4** (red trace), **5** (blue trace), and **6** (cyan trace). Inset: Expansion of the pre-edge region.

Table 5.1: XANES Data for complexes **1**, **2**, **3**, **4**, **5**, and **6**.

Complex	Edge Energy (eV)	Pre-Edge Area (units)	Pre-Edge Peak (eV)
1	6548.4 (± 0.1)	8.8 (± 0.1)	6540.4 (± 0.1)
2	6548.9 (± 0.1)	13.5 (± 0.1)	6540.6 (± 0.1)
4	6549.4 (± 0.1)	10.2 (± 0.1)	6540.5 (± 0.1)
5	6549.0 (± 0.1)	8.7 (± 0.1)	6540.5 (± 0.1)
6	6549.2 (± 0.1)	14.5 (± 0.1)	6540.7 (± 0.1)

With the combination of UV-vis, EPR, X-ray absorption spectroscopies, and mass spectrometry studies we assigned the species **6** to be a new bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex with the possible association of a Sc^{3+} ion (Figure 5.8). This discovery was very interesting for the catalytic cycle of Ib RNRs as this is the third bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex generated from a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (Chapters 3 and 4). Therefore, formation of a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ structure appeared to be thermodynamically favoured by this $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**2**). This lends further support that the unknown intermediate in the catalytic cycle of Ib RNRs was a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide as this species was capable of easily converting to a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species, the active oxidant in the catalytic cycle responsible for tyrosine oxidation.¹

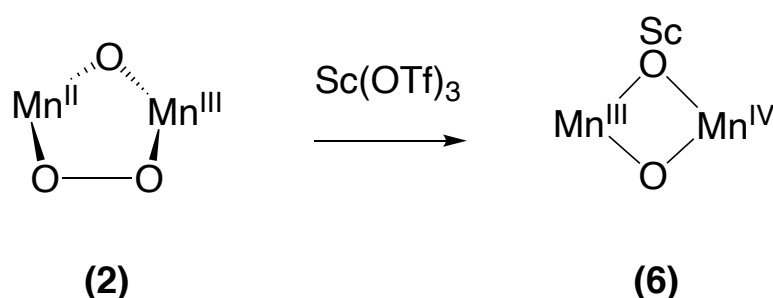


Figure 5.8: Formation of **6** following the reaction of **2** + 1 eq. Sc(OTf)_3 .

5.3 Reactivity

Complex **6** was postulated to possess a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ structure with a possible Sc^{+3} associated to the diamond core. Therefore, we were interested in probing the reactivity of this new species. The phenol substrate 4-methoxy-2,6-di-*tert*-butylphenol (4-OCH₃-2,6-DTBP) was chosen to act as a functional mimic for the tyrosine residue in the catalytic cycle of Ib RNRs. Addition of 50 eq. of 4-OCH₃-2,6-DTBP to **6** at -90 °C was monitored via UV-vis spectroscopy (Figure 5.9, left). Upon addition of the phenol substrate, decay of

the band at $\lambda_{\text{max}} = 550$ nm associated with **6** was observed with the concurrent formation of a new sharp band at $\lambda_{\text{max}} = 405$ nm. This sharp band was associated with the formation of the 4-methoxy-2,6-di-*tert*-butylphenoxyl radical species as it has been observed previously in the reaction of metal complexes and 4-OCH₃-2,6-DTBP.¹⁹ To confirm the postulated radical species, EPR spectroscopy of the post-reaction mixture was performed at 77 K and revealed an intense, isotropic signal centred at $g = 2$ (Figure 5.9, right). The isotropic signal was simulated as a $S = \frac{1}{2}$ signal centred at $g = 2$. This signal was indicative of the formation of an organic radical species. With the combination of characteristic UV-vis and EPR data, we assigned 4-methoxy-2,6-di-*tert*-butylphenoxyl radical to be the product formed following reaction of **6** with 4-OCH₃-2,6-DTBP.

Interestingly, no metal-based signals were identifiable in the EPR spectrum of the post-reaction mixture of **6** + 4-OCH₃-2,6-DTBP. Although organic based EPR signals are far more intense than the corresponding metal based ones, they are also far narrower. Despite this, no Mn based signals were identifiable in the post-reaction EPR. Hence, we postulated that following reaction with 4-OCH₃-2,6-DTBP, **6** converted to a EPR silent species. This was analogous to the catalytic cycle of Ib RNRs whereby following reaction with a tyrosine residue the Mn^{III}Mn^{IV} active species converted to a EPR silent Mn^{III}Mn^{III} complex. Therefore, we postulated that **6** also converted to an EPR silent Mn^{III}Mn^{III} complex following reaction with 4-OCH₃-2,6-DTBP.

Interestingly, the other two Mn^{III}Mn^{IV} complexes studied in this thesis (Complexes **4** and **5**, Chapters 3 and 4) also appeared to convert to EPR silent species following reaction with the substrate 4-OCH₃-2,6-DTBP. Therefore, all of these Mn^{III}Mn^{IV} complexes followed a common reactivity. It appeared that this reactivity path was thermodynamically favourable among the various Mn^{III}Mn^{IV} complexes. This was of particular relevance to the catalytic cycle of Ib RNRs as it provided further weight that a Mn^{III}Mn^{IV} species reacted with the O-

H bond of the tyrosine residue and formed an EPR silent $\text{Mn}^{\text{III}}\text{Mn}^{\text{III}}$ species and tyrosyl radical species.

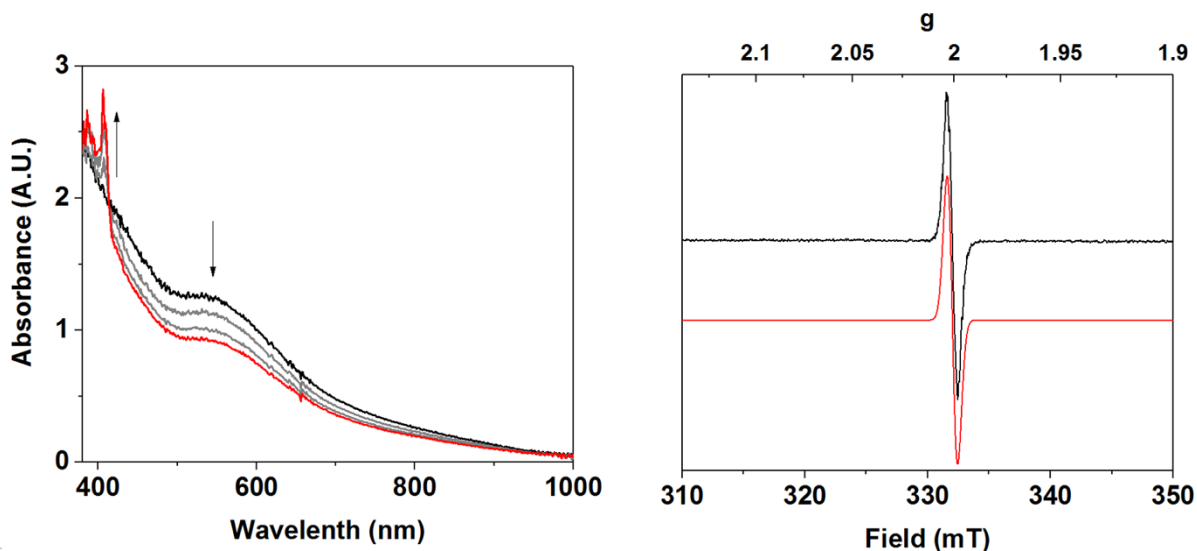


Figure 5.9: Left: UV-vis absorption spectra of the reaction of **6** (black trace) + 50 eq. 4-OCH₃-2,6-DTBP (red trace) in 1:9 MeCN/THF at -90 °C. Right: Perpendicular X-Band EPR spectrum of the reaction of **6** and 4-OCH₃-2,6-DTBP measured at 77 K (9.65 GHz, 0.2 mW microwave power) in black and the simulated spectra in red.

We were interested in investigating if the reactivity observed between **6** and 4-OCH₃-2,6-DTBP could be replicated using other O-H bond containing substrates. Therefore, **6** was reacted with three substrates from the 1-hydroxy-2,2,6,6-tetramethyl-piperidine (TEMPO-H) family. Addition of these substrates to **6** resulted in the decay in the electronic absorption features associated with the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species (A5.9 – A5.11). EPR spectroscopy of the post-reaction mixtures of **6** + 4-X-TEMPO (where X = OCH₃, O, and H) all revealed an intense rhombic signal with $g_x = 2.02$, $g_y = 2.01$, and $g_z = 1.98$ (A5.12 – A5.14). An intense rhombic signal with $g_{av} = 2$ was characteristic of the formation of the corresponding TEMPO· radical species and has been extensively documented in literature.¹⁴ The yields of the radical species were quantified via EPR using a TEMPO· radical standard, assuming an one-electron oxidation (Table 5.2). Therefore, we determined that **6** was capable of reacting with a variety of O-H bonds of varying strength.

Table 5.2: Yields of the organic radical species determined via EPR following reaction of **6** + substrate.

Substrate	EPR Radical Yield ($\pm 15\%$)	BDFE (kcal mol ⁻¹)
4-OCH ₃ -TEMPO-H	50 %	65
4-Oxo-TEMPO-H	50 %	65.6
TEMPO-H	55 %	66.5
4-OCH ₃ -2,6-DTPB	15 %	74.9

To further probe the kinetics of new complex **6**, its reactivity with 4-OCH₃-TEMPO-H was analysed under *pseudo* first-order conditions. Excess substrate was added to **6** and the decay of the complex was monitored at $\lambda_{\text{max}} = 550$ nm. Fitting this decay afforded a *pseudo* first-order rate constant (k_{obs}) for that substrate concentration. A plot k_{obs} versus [4-OCH₃-TEMPO-H] was constructed and revealed a linear relationship between these parameters (Figure 5.10). A second-order rate constant (k_2) of 0.96 M⁻¹ s⁻¹ was obtained from the slope of the linear fit of this plot (Figure 5.10).

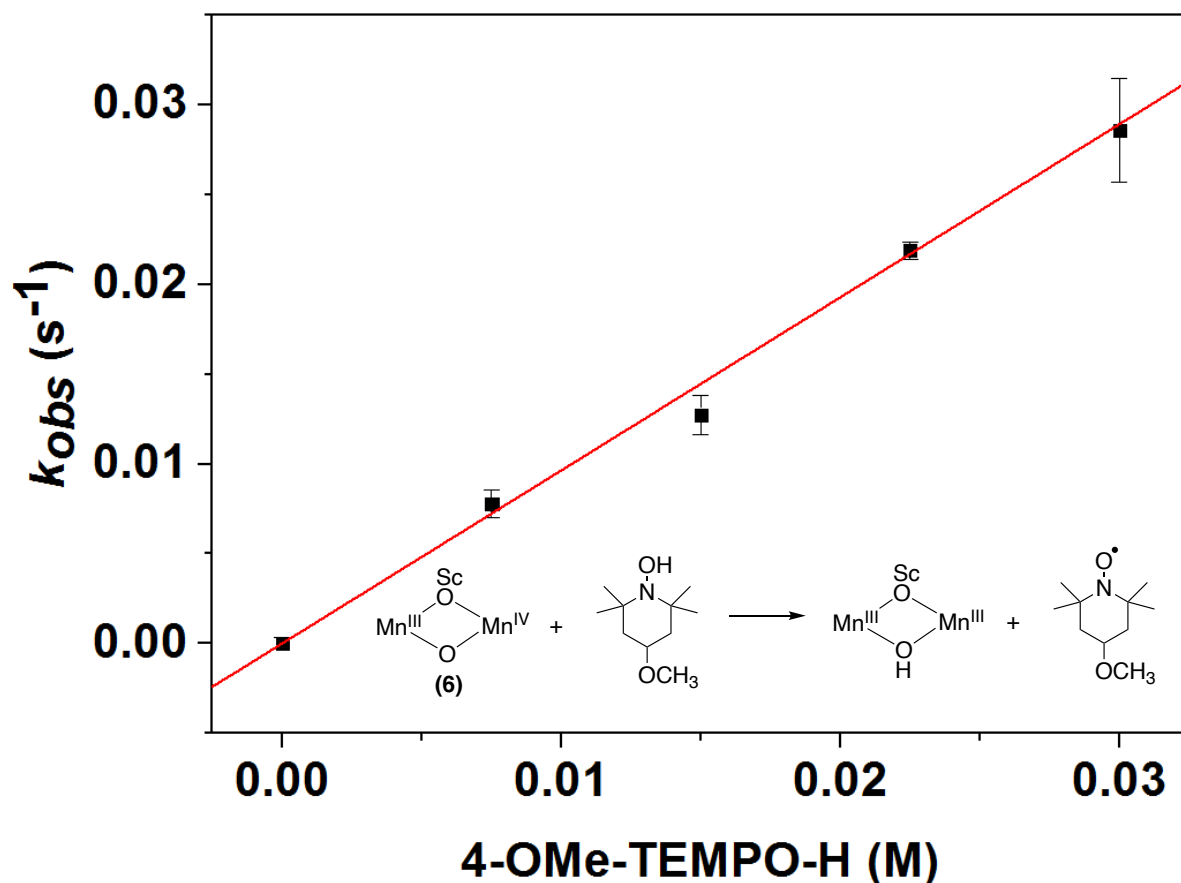


Figure 5.10: Slope = 0.96 ($R^2 = 0.997$) Inset: Scheme of the reaction of **6** and 4-OCH₃-TEMPO-H.

This plot was very revealing of the reactivity of new species **6**. Firstly, **6** appeared to react with O-H bonds via a linear mechanism. This was interesting as complex **4** (Chapter 3) also reacted with O-H bonds via a linear mechanism, however, complex **5** (Chapter 4) displayed saturation behaviour implying a Michaelis-Menten mechanism. The difference between complexes **4** and **6** versus **5** was that we postulated the cores of **4** and **6** contained a Lewis acid species - a proton (H^+) in the case of **4**, and Sc^{3+} for **6** (Figure 5.11). Whereas complex **5** contained a “naked” bis(μ -oxo) $Mn^{III}Mn^{IV}$ core (Figure 5.11). This further supported our postulate that the bis(μ -oxo) $Mn^{III}Mn^{IV}$ cores required “activation” in order to be reactive enough to oxidise O-H bonds. The Lewis acids on the cores of **4** and **6** increased the electrophilicity of the cores and allowed the complexes to directly react with the O-H bonds and resulted in a linear relationship between k_{obs} and [substrate]. However, the complex **4**

contained a “naked” bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core, which was not reactive enough to oxidise an O-H bond. Therefore, the proton of the O-H bond must associate with bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core in order to “activate” it towards reactivity. This resulted in the saturation behaviour observed in the plot of k_{obs} versus [substrate].

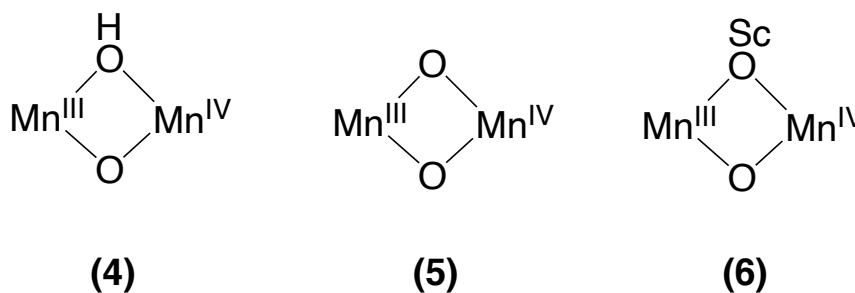


Figure 5.11: Structure of complexes 4, 5, and 6.

The k_2 for the reaction between **6** and 4-OCH₃-TEMPO-H was determined to be $0.96 (\pm 0.03) \text{ M}^{-1} \text{ s}^{-1}$. Interestingly, the k_2 was calculated to be $0.26 (\pm 0.01) \text{ M}^{-1} \text{ s}^{-1}$ for the reaction of the (μ -hydroxo)(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex (**4**) with the same substrate, 4-OCH₃-TEMPO-H. The k_2 of both complexes were recorded under identical conditions in the same solvent system at -90°C . This represented a four-fold increase in the rate of the reaction when complex **6** was employed instead of **4**. This was of note because the only difference between these two complexes was the identity of the species associated to the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core of the complex. We postulated that H^+ and Sc^{3+} species are associated to the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core of **4** and **6** respectively and are the source of the variation in reactivity of the complexes. Sc^{3+} is a stronger Lewis acid in comparison to H^+ . Therefore, the presence of Sc^{3+} in **6** made the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core more electrophilic than that of **4** and hence was the source of the increased rate (Figure 5.12). This further confirmed our postulate that the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ core must be activated to react with O-H bonds, and using Lewis acids of increased strength increases the rate of the reaction.

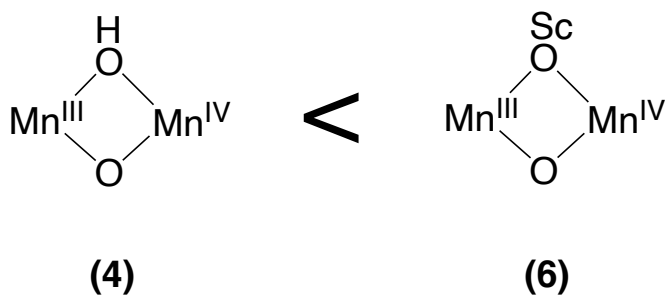


Figure 5.12: Complex **6** reacts faster with O-H bonds compared to complex **4** due to the presence of a stronger Lewis acid.

5.4 Conclusions

Upon addition of 1 eq. of Sc(OTf)₃ to the Mn^{II}Mn^{III}-peroxide complex (**2**) an immediate conversion to a new species (**6**) was observed via UV-vis spectroscopy. Further investigations of this new species via EPR, XAS, and mass spectrometry techniques revealed **6** to be a new bis(μ-oxo)Mn^{III}Mn^{IV} species with the possible association of a Sc³⁺ ion to the oxo-core.

The reactivity of **6** towards O-H bonds was investigated via UV-vis and EPR spectroscopies. **6** was determined to be capable of the one-electron oxidation of a variety of substrates including 4-OCH₃-2,6-DTBP and 4-X-TEMPO-H (where X = H, OCH₃, O). The second-order rate constant (*k*₂) for the reaction of **6** with the substrate 4-OCH₃-TEMPO-H was calculated to be 0.96 M⁻¹ s⁻¹. **6** reacted much faster with the same substrate in comparison to a related (μ-hydroxo)(μ-oxo)Mn^{III}Mn^{IV} species studied previously (**4**, Chapter 3). We postulated that the enhanced rate displayed by **6** was due to the presence of Sc³⁺ on the oxo-core, which increased the electrophilicity of the diamond core and thus increased the reactivity of **6**.

5.5 Experimental

Materials

All reactions with air-sensitive materials were performed in a glove box in a N₂ atmosphere or under inert conditions using inert Schlenk line techniques. All reagents and solvents were purchased from commercial sources. Anhydrous *N,N*-dimethylformamide (DMF) was purchased and used without further purification. Anhydrous tetrahydrofuran (THF) and acetonitrile (MeCN) were dispensed through an MBraun MB-SPS-5 Solvent Purification System and deoxygenated by purging with argon.

N,N-bis(2-picoyl)amine and 2,6-bis(chloromethyl)-4-methylphenol were synthesised as previously described.^{20,21} The ligand 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol (HBPMP) was synthesised as previously reported.²² The synthesis of [Mn₂(O₂CCH₃)₂(BPMP)](ClO₄), **1**, was carried out as previously reported.²³

Caution: KO₂ and perchlorate salts of metal complexes are potentially explosive and must be handled with care and in small quantities. No problems were encountered during this project.

Physical Methods

¹H NMR analysis was performed on a Bruker Avance III 400 MHz instrument. Electrospray ionisation (ESI) mass spectra were obtained using micromass time of flight spectrometer (TOF), interfaced to a Waters 2690 HPLC, or by direct injection in the mass spectrometry instrument. Electronic absorption spectra were recorded in quartz cuvettes on a Hewlett Packard (Agilent) 8453 diode array spectrophotometer (190 – 1100 nm range) coupled to a liquid nitrogen cooled cryostat from Unisoku Scientific Instruments (Osaka, Japan). Electronic paramagnetic resonance (EPR) measurements were conducted on a Bruker Elexsys E-500 spectrometer with an Oxford ESR 910 liquid helium cryostat and an

Oxford temperature controller. The 30 K EPR quantification of the signals was relative to a $[\text{Cu}(\text{NO}_3)_2]$ spin standard in CH_3OH . The 77 K EPR quantification of the signals were relative to a TEMPO $\cdot$ spin standard in 1:9 MeCN/THF. EPR spectra of 4-X-TEMPO and ABNO were recorded at 77 K, 9.2 GHz, 2.02 mW microwave power, with a 60 mT field sweep in 84 s, and 0.3 mT field modulation amplitude. Integration, simulation, and fitting were performed with Matlab and the easySpin computational package.²⁴ The simulation for the phenoxyl radical spectra was modelled as an $S = \frac{1}{2}$ electron spin with an isotropic g tensor.

X-ray absorption spectroscopy methods: The Mn-K-edge X-ray absorption data were collected on beamline 7-3 of the Stanford Synchrotron Radiation Lightsource (SLAC National Accelerator Lab, Menlo Park, CA, USA). Data were collected with the storage ring operating at 3.0 GeV and 500 mA, using a LN2 cooled Si(220), $\phi = 90^\circ$ double-crystal monochromator, calibrated by using the first inflection point of a Mn foil (6539.0 eV). The monochromator was detuned by $\sim 50\%$ for higher harmonic rejection. A Canberra 32-element solid state germanium detector was used for fluorescence detection. All the measurements were performed at ambient pressure at ~ 17 K using an Oxford helium cryostat that was cooled by closed-cycle He gas loop. The parameters used for the scans were the following: 10 eV steps/1 second integration time in the pre-edge region, 0.3 eV steps /2 second integration time in the edge, and 0.05k steps in the EXAFS, with integration time increasing in a k^2 -weighted fashion from 2 to 4 seconds over the energy range ($k_{\text{max}} = 12.1k$). The total detector counts were typically 3-7k, well within the linear range of the detector electronics. Each sample was monitored for photoreduction. Complex **2** was found to be photoreduced by the X-rays and so only one scan was obtained per spot. Delrin ® Mossbauer/XAS cups with sample windows of 4 mm x 10 mm. Elaboration of the XAS data, including averaging, background removal and normalization, was performed using

Athena.²⁵ Edge energies were obtained by taking the first derivative of the rising edge, while pre-edge energies were identified using the second derivative. Peak fitting analysis employed the program Fityk,²⁶ using the protocol developed by Westre et al. for analysing Fe K-edge pre-edge peaks.²⁷ Briefly, a 15-18 eV segment of the normalized $\mu(E)$ spectrum was fit around the pre-edge, with pre-edge peaks modelled using pseudo-Voigt functions having a fixed 50:50 mixture of Gaussian: Lorentzian character, while the background absorption was modelled with a single pseudo-Voigt function where the proportion of Gaussian: Lorentzian character was allowed to float. Four slightly different energy ranges were fit separately, and the results averaged to generate the reported peak fits.

Experimental Procedures

General procedure for the reaction of 1 with KO₂ and cryptand

A 1:9 CH₃CN/THF solution (1.5 mM) of complex **1** was prepared. In a quartz cuvette 2 mL of a 1.5 mM solution of complex **1** was cooled to -90 °C. KO₂ (0.0056 g, 0.04 M) and cryptand (0.06 g, 0.08 M) were dissolved in DMF (2 mL). 75 μ L of this solution was added to the solution containing **1**. The reaction progress was monitored using electronic absorption spectroscopy. Lower yields were observed (less intense absorption features) with high equivalencies of KO₂ and at higher concentrations of **1**.

General Procedure for the reaction of 2 with Sc(OTf)₃

A solution of **2** was prepared in a quartz cuvette at -90 °C as outlined in the procedure above. Sc(OTf)₃ (0.09 g, 0.18 M) was dissolved in 1 mL 1:9 MeCN/THF. 16 μ L of this solution was added to the solution containing **2**. The reaction progress was monitored using electronic absorption spectroscopy.

EPR Sample Preparation

Samples for EPR analysis were prepared by transferring ~ 1 mL of the desired solution from the quartz cuvette at -90 °C, via a pre-cooled pipette into a pre-cooled EPR tube and immediately freezing it in liquid nitrogen. The EPR spectra were recorded at 2 K and 77 K (9.64 GHz, 0.2 mW microwave power).

Frozen Mass Spectrometry Sample Preparation

The samples for frozen MS analysis were prepared by transferring ~ 0.1 mL of the desired solution from the quartz cuvette, pre-cooled to -90 °C, via a pre-cooled MS sample vial with pre-cooled methanol and immediately freezing in liquid nitrogen. ESI- MS was performed on the just cooled sample.

Reactivity Studies

A solution of **2** in 1:9 MeCN/THF at -90 °C was prepared using the above procedure with Cryptand. Substrates were added as concentrated MeCN/THF solutions to solutions of **2** at -90 °C. The reactions were monitored using electronic absorption spectroscopy.

Preparation of $K^{18}O_2$:

Method was adapted from a similar procedure reported by Tolman *et al.*²⁸ TEMPO-H (0.159 g, 1 mmol) was reacted with potassium hydride (KH) in 2 ml of THF. Solution was allowed to stir for two hours until gas evolution ceased. Solution was filtered through a 0.2 μ m syringe filter and the filter washed with an addition 2 ml THF. Solution freeze pump thawed to ensure an environment free of $^{16}O_2$. The solution was frozen in liquid nitrogen and the headspace evacuated and refilled with $^{18}O_2$. Solution was allowed to warm to room temperature and upon this changed from a colourless solution to a dark orange colour. The

solvent was removed under vacuum resulting in a dark orange residue. The residue was washed with dry, degassed hexane to yield a yellow precipitate. The hexane was decanted and the powder was washed several times with hexane until the decanted liquid was completely colourless. The yellow powder was then dried under vacuum and used without further purification.

5.6 References

- (1) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, *135* (10), 4027–4039.
- (2) Banerjee, S.; Draksharapu, A.; Crossland, P. M.; Fan, R.; Guo, Y.; Swart, M.; Que, L. *J. Am. Chem. Soc.* **2020**, *142* (9), 4285–4297.
- (3) Horner, O.; Anxolabéhère-Mallart, E.; Charlot, M. F.; Tchertanov, L.; Guilhem, J.; Mattioli, T. A.; Boussac, A.; Girerd, J. J. *Inorg. Chem.* **1999**, *38* (6), 1222–1232.
- (4) Howard, T.; Telser, J.; DeRose, V. J. *Inorg. Chem.* **2000**, *39* (15), 3379–3385.
- (5) Golombek, A. P.; Hendrich, M. P. *J. Magn. Reson.* **2003**, *165* (1), 33–48.
- (6) Diril, H.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S.; Chang, H. R.; Nilges, M. J.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, *111* (14), 5102–5114.
- (7) Lessa, J. A.; Horn, A.; Bull, É. S.; Rocha, M. R.; Benassi, M.; Catharino, R. R.; Eberlin, M. N.; Casellato, A.; Noble, C. J.; Hanson, G. R.; Schenk, G.; Silva, G. C.; Antunes, O. A. C.; Fernandes, C. *Inorg. Chem.* **2009**, *48* (10), 4569–4579.
- (8) Yan Poon, P. C.; Dedushko, M. A.; Sun, X.; Yang, G.; Toledo, S.; Hayes, E. C.; Johansen, A.; Piquette, M. C.; Rees, J. A.; Stoll, S.; Rybak-Akimova, E.; Kovacs, J. A. *J. Am. Chem. Soc.* **2019**, *141* (38), 15046–15057.
- (9) Lee, Y.; Jackson, T. A. *ChemistrySelect* **2018**, *3* (47), 13507–13516.
- (10) Hagen, K. S.; Armstrong, W. H.; Hope, H. *Inorg. Chem.* **1988**, *27* (6), 967–969.
- (11) Brewer, K. J.; Calvin, M.; Lumpkin, R. S.; Otvos, J. W.; Spree, L. O. *Inorg. Chem.* **1989**, *28* (25), 4446–4451.
- (12) Schäfer, K. O.; Bittl, R.; Zweggart, W.; Lendzian, F.; Haselhorst, G.; Weyhermüller, T.; Wieghardt, K.; Lubitz, W. *J. Am. Chem. Soc.* **1998**, *120* (50), 13104–13120.
- (13) Magherusan, A. M.; Kal, S.; Nelis, D. N.; Doyle, L. M.; Farquhar, E. R.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2019**, *58* (17), 5718–5722.
- (14) Lovisari, M.; McDonald, A. R. *Inorg. Chem.* **2020**, *59* (6), 3659–3665.
- (15) Schreiber, R. E.; Cohen, H.; Leitus, G.; Wolf, S. G.; Zhou, A.; Que, L.; Neumann, R. *J. Am. Chem. Soc.* **2015**, *137* (27), 8738–8748.
- (16) Weng, T. C.; Hsieh, W. Y.; Uffelman, E. S.; Gordon-Wylie, S. W.; Collins, T. J.; Pecoraro, V. L.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **2004**, *126* (26), 8070–8071.
- (17) Rose, H. R.; Ghosh, M. K.; Maggiolo, A. O.; Pollock, C. J.; Blaesi, E. J.; Hajj, V.; Wei, Y.; Rajakovich, L. J.; Chang, W. C.; Han, Y.; Hajj, M.; Krebs, C.; Silakov, A.; Pandelia, M. E.; Bollinger, J. M.; Boal, A. K. *Biochemistry* **2018**, *57* (18), 2679–2693.
- (18) Stemmler, T. L.; Sturgeon, B. E.; Randall, D. W.; Britt, R. D.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **1997**, *119* (39), 9215–9225.

- (19) Spedalotto, G.; Lovisari, M.; McDonald, A. R. *ACS Omega* **2021**, 6 (42), 28162–28170.
- (20) Borovik, A. S.; Que, L.; Papaefthymiou, V.; Taylor, L. F.; Anderson, O. P. *J. Am. Chem. Soc.* **1989**, 111 (16), 6183–6195.
- (21) Raycroft, M. A. R.; Maxwell, C. I.; Oldham, R. A. A.; Andrea, A. S.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2012**, 51 (19), 10325–10333.
- (22) Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, 39 (16), 3526–3536.
- (23) Suzuki, M.; Mikuriya, M.; Murata, S.; Uehara, A.; Oshio, H.; Kida, S.; Saito, K. *Bull. Chem. Soc. Jpn.* **1987**, 60 (12), 4305–4312.
- (24) Stoll, S.; Schweiger, A. *J. Magn. Reson.* **2006**, 178 (1), 42–55.
- (25) Ravel, B.; Newville, M. *J. Synchrotron Radiat.* **2005**, 12 (4), 537–541.
- (26) Wojdyr, M. *J. Appl. Crystallogr.* **2010**, 43 (5), 1126–1128.
- (27) Westre, T. E.; Kennepohl, P.; DeWitt, J. G.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1997**, 119 (27), 6297–6314.
- (28) Donoghue, P. J.; Gupta, A. K.; Boyce, D. W.; Cramer, C. J.; Tolman, W. B. *J. Am. Chem. Soc.* **2010**, 132 (45), 15869–15871.

Chapter 6: Synthesis of a New Dinuclear Mn₂^{II} Complex and its' Reactivity with Superoxide.

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6.0 Abstract

A new dinuclear manganese complex $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$ (**7**), where TPDP = 1,3-bis(bis(pyridin-2-ylmethyl)amino)propan-2-ol, was synthesised and fully characterised via single-crystal X-ray diffraction, X-ray absorption, infrared spectroscopies, and mass spectrometry. **7** reacted with superoxide (O_2^-) and generated a new species **8**. The UV-vis and EPR spectra of **7** was remarkably similar to previously published $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species. Further X-ray near edge absorption spectroscopy (XANES) studies confirmed the assignment of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide.

Treatment of this new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, **8**, with two equivalents of the acid *para*-toluenesulfonic acid (pTsOH) resulted in the conversion to a new species **9**. EPR experiments performed on **9** showed a 21-line signal centred at $g \approx 2$ and a low-field signal at $g = 6.05$ associated with high spin transitions. These spectral features were characteristic of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -species, and crucially these features were distinct from the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ features of **8**. The XANES edge energy of **9** was also representative of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -species. The pre-edge area of **9** was substantially smaller than that of **8**. This decrease of area has been observed before for Mn-hydroperoxo species formed following addition of an acid to a Mn^{III} -peroxide. Therefore, we assigned **9** to be the first $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species to be characterised by UV-vis, EPR, and X-ray absorption spectroscopies.

6.1 Introduction

Ribonucleotide reductase (RNRs) are metalloenzymes that catalyse the conversion of ribonucleotides to the corresponding deoxyribonucleotides.¹ This represented the initial and rate limiting step in deoxyribonucleic acid (DNA) synthesis and repair in all organisms. RNRs are divided into five subclasses depending on the metals in their active site, their activating oxidants, and the radical species generated during the catalytic cycle.² The subclass Ib RNRs contained a dinuclear manganese cofactor, was activated using superoxide (O_2^-) as an oxidant, and generated a tyrosyl radical species upon oxidation of a nearby tyrosine.

In the proposed catalytic cycle of Ib RNRs, O_2 reacts with a nearby flavodoxin protein (NrdI_{hq}) to form superoxide (O_2^-) (Figure 6.1).³ O_2^- then reacted with the dinuclear manganese centres and was postulated to form a $Mn^{II}Mn^{III}$ -peroxide species that rapidly interconverted, possibly with the involvement of a proton to a bis(μ -oxo) $Mn^{III}Mn^{IV}$. This $Mn^{III}Mn^{IV}$ species was the active oxidant in the catalytic cycle and was responsible for the one-electron oxidation of a nearby tyrosine to a tyrosyl radical. Formation of the tyrosyl radical initiated ribonucleotide reduction.

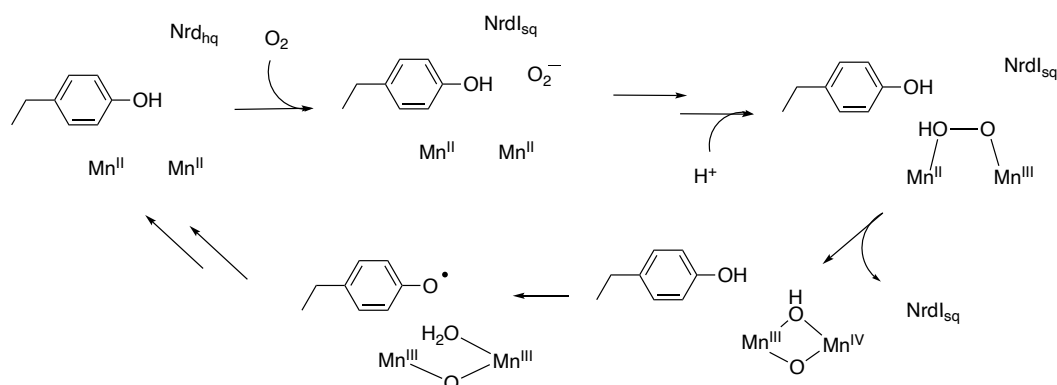


Figure 6.1: Postulated catalytic cycle for class Ib RNRs.

Ib RNRs have been intensely studied for a number of years, yet despite this, many questions still linger around its catalytic cycle. For example, the postulated $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -(hydro)peroxide intermediate has never been trapped and no spectroscopic evidence exists of its structure. Furthermore, only one electron paramagnetic resonance (EPR) spectrum provided evidence for the formation of a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species responsible for tyrosine oxidation.³

Biomimetic complexes that replicate the environment of the active site of enzymes offer an opportunity to study the reactivity of enzymes while subverting the problems typically associated with studying the enzymes directly. For example, in 2018 McDonald *et al.* reported a dinuclear manganese complex, $([\text{Mn}^{\text{II}}_2(\text{OCCH}_3)(\text{N-Et-HPTB})](\text{ClO}_4)_2)$, where N-Et-HPTB = N,N,N',N'-tetrakis(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3-diaminopropane, capable of mimicking Ib RNRs reactivity with superoxide to form a mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide characterised by UV-vis, EPR, and X-ray absorption spectroscopies and mass spectrometry (Figure 6.2).⁴ The first $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide synthesised by the McDonald group, $[\text{Mn}_2(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$, was supported by a polybenzimidazole ligand and was unreactive at $-40\text{ }^\circ\text{C}$.⁴ Another $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species ($\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})$, Chapter 2), was supported by a polypyridine ligand and displayed nucleophilic reactivity at $-90\text{ }^\circ\text{C}$.⁵ This represented some of the first experimental support for the formation of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species in the catalytic cycle of Ib RNRs. We seek to build on these discoveries and develop new dinuclear manganese complexes in order to probe their reactivity with superoxide and if changes in ligand environment will influence any new species formed.

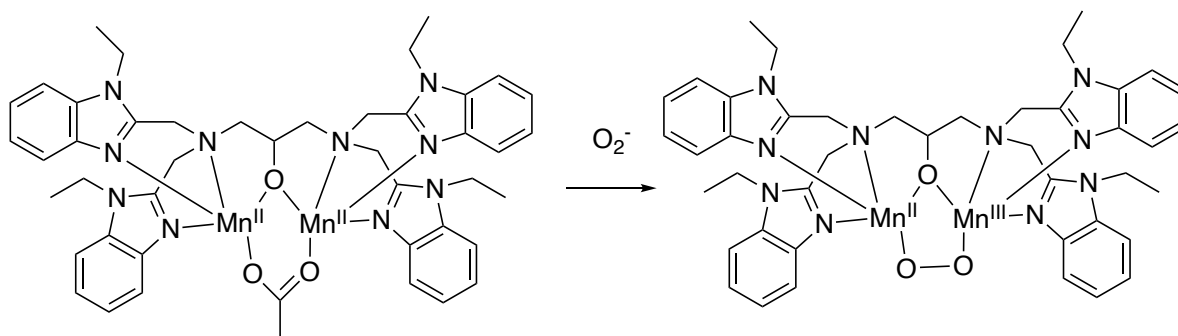


Figure 6.2: Reaction of $[\text{Mn}^{\text{II}}_2(\text{OCCH}_3)(\text{N-Et-HPTB})](\text{ClO}_4)_2$ with superoxide to form a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species.

In order to develop more reactive $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species, the polypyridine ligand, TPDP (TPDP = 1,3-bis(bis(pyridin-2-ylmethyl)amino)propan-2-ol) was selected. The TPDP ligand contained an aliphatic backbone and polypyridine moieties (Figure 6.3), which we postulated will provide greater access to the Mn^{II}_2 centres and enhance the reactivity of the complex in comparison to the previously studied $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$.⁴ Moreover, pyridine ligands are good sigma donors and π -acceptors and we postulated that the increased electron density may allow higher Mn oxidation states to be accessed.^{6,7}

6.2 Results and Discussion

TPDP was synthesised via a previously reported procedure and its structure and purity confirmed via nuclear magnetic resonance (NMR) spectroscopy (A6.1).⁸ Synthesis of $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$ (**7**) was adapted from the synthesis of an analogous iron complex (Figure 6.3).⁹ The synthesis of **7** was performed under inert conditions and involved ion exchange with sodium tetraphenylborate to initiate precipitation of the complex, see details in experimental section (6.8).

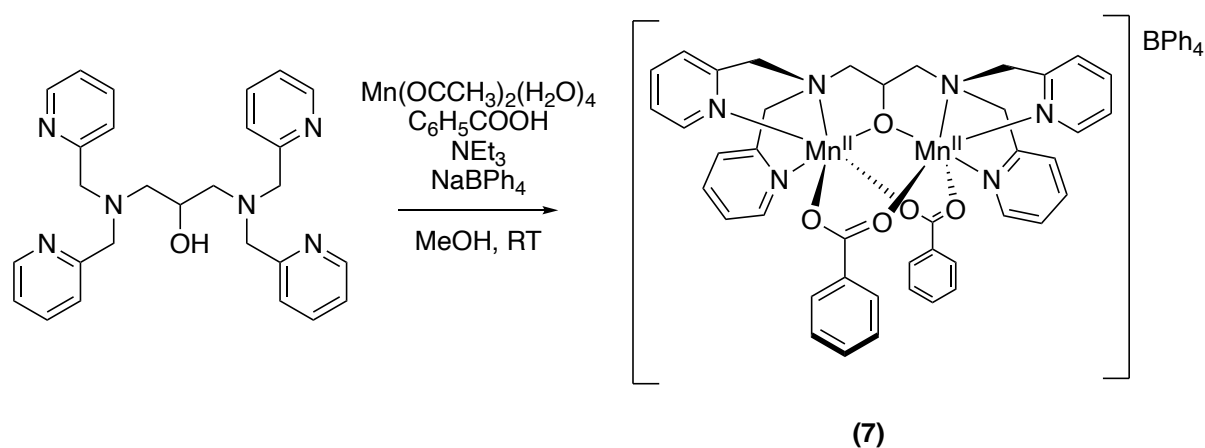


Figure 6.3: Synthesis of $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$ (**7**).

The product of the reaction was a yellow/brown powder and was recrystallised from acetonitrile (MeCN) at -35°C under inert conditions to give yellow crystals that were analysed via single-crystal X-ray diffraction analysis (XRD) (Figure 6.4). In the molecular structure of **7** two distorted octahedral Mn^{II} centres were observed. This distortion mainly originated from the O1-Mn1-N1 and O1-Mn2-N2 angles of $75.57(4)^\circ$ and $76.58(4)^\circ$ which deviated from the ideal value of 90° (Table 6.1). Excluding the counter ion, **7** contained an almost symmetrical geometry with a pseudo mirror plane cutting through the O1 atom and the benzoate moieties. Another source of asymmetry found in complex **7** was the asymmetric binding of the benzoate moieties, illustrated by the Mn1-O4 and Mn2-O3

bonds which were on average 0.04 Å shorter than the Mn1-O2 and Mn2-O5 bonds (Table 6.1). This type of unsymmetric binding of carboxylate groups was observed before in the crystal structure of $[\text{Mn}^{\text{II}}_2(\text{BPMP})(\text{OCCH}_3)_2](\text{ClO}_4)$.¹⁰ The bonds of the Mn centres 1 and 2 to the O1 atom of the alkoxygroup on the ligand were unusually short at just 2.05 and 2.06 Å (Table 6.1). This was unexpected as this oxygen atom was coordinated by three atoms. In contrast, the oxygen atoms of the benzoic acid moieties are only coordinated by two atoms by display longer O-Mn bonds between 0.05 and 0.10 Å. This emphasised the strong interaction between the Mn centres and the ligand backbone.

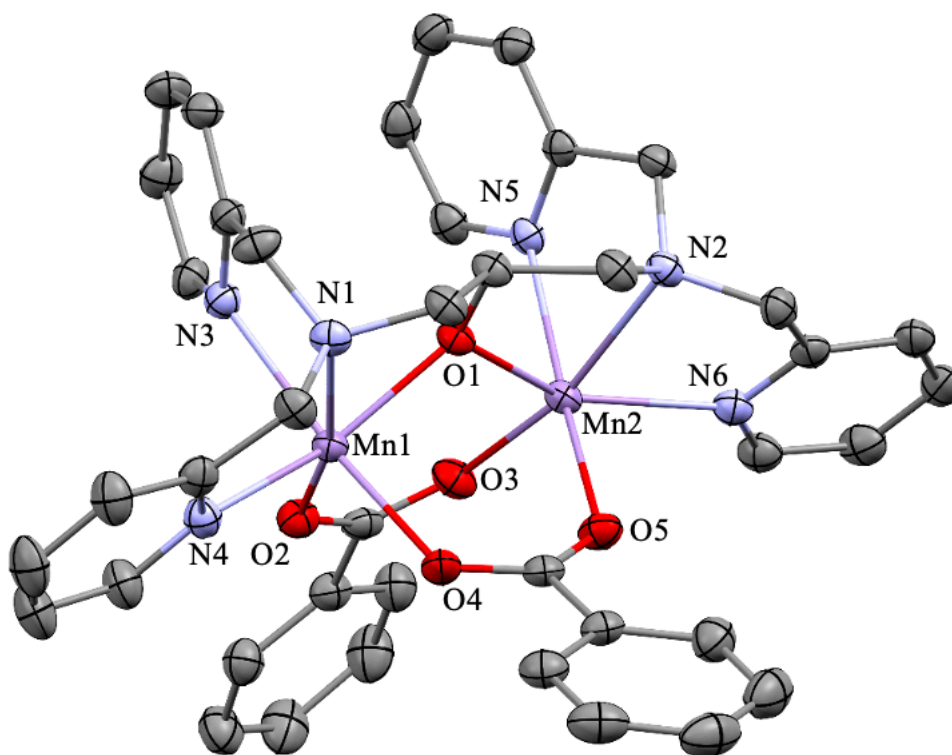


Figure 6.4: Molecular structure of **7** measured by XRD. Hydrogen atoms, BPh_4 counterion, and two MeCN solvent molecules were omitted for clarity. Ellipsoids are 50% probability.

Table 6.1: Selected bond lengths, angles and dihedral angles of complex 7.

Atoms	Bond length (Å)/Angle (°)
Mn1···Mn2	3.3782(3)
Mn1–O1	2.0527(10)
Mn2–O1	2.0608(11)
Mn1–N1	2.3655(12)
Mn2–N2	2.3125(13)
Mn1–N3	2.2819(13)
Mn1–N4	2.2727(13)
Mn2–N5	2.3084(13)
Mn2–N6	2.2637(14)
Mn1–O2	2.1517(11)
Mn1–O4	2.1254(11)
Mn2–O3	2.1046(11)
Mn2–O5	2.1675(11)
O1–Mn1–N1	75.57(4)
O1–Mn2–N2	76.58(4)
Mn2–N2–N1–Mn1	0.684(6)

7 displayed very similar geometry in comparison to the dinuclear manganese complex $[\text{Mn}^{\text{II}}_2(\text{BPMP})(\text{OCCH}_3)_2](\text{ClO}_4)$ (**1**, Chapter 2, Table 6.2). For example, the Mn-Mn separation in complexes **1** and **7** were both within error of each other at 3.41 and 3.38(3) Å respectively.¹⁰ Both **1** and **7** contained Mn-Mn distances much shorter than that found in the native Ib RNR enzymes. For instance, in the crystal structure of Ib RNR *Bacillus subtilis* there was an Mn-Mn separation of 3.7 Å, approximately 0.3 Å longer than that of **1** and **7**. An even greater Mn-Mn distance of 3.9 Å was found in the crystal structure of *Escherichia coli*, approximately 0.5 Å longer than **1** and **7**. This can be easily rationalised as **1** and **7** contained chelating ligands that forced the Mn centres into a closer geometry, while the Mn centres of the Ib RNR enzymes were ligated by several amino acids, that provided the protein much more flexibility.¹¹

The trend of similarity for **1** and **7** was repeated for other distances in the crystal structure, for example, the Mn1-N1 and Mn1-N2 distances were also within error of each other for both complexes (Table 6.2). Interestingly, the Mn-N distances of complexes **1** and **7** were

closer in agreement to the Mn-N values of found in the enzymes (Table 6.2). A difference of only ~ 0.1 Å between the Mn-N lengths of the synthetic complexes and the native enzymes was observed. Both **1** and **7** utilised pyridines to mimic the Mn-N environment of the enzyme and the similarity of bond lengths they displayed with the enzymes reinforced that these moieties were good mimics for the environment of the enzyme.

Table 6.2: Comparison of selected bond lengths (in Å) of **1**, **7**, and *Ib* RNRs.

Atoms	[Mn ^{II} ₂ (O ₂ CC ₆ H ₅) (TPDP)] ⁺ (7)	[Mn ^{II} ₂ (OCCH ₃) (BPMP)] ⁺ (1) ¹²	<i>B. subtilis</i> <i>Ib</i> RNR ¹³	<i>E. Coli</i> <i>Ib</i> RNR ¹⁴
Mn1...Mn2	3.3782(3)	3.41(1)	3.7	3.9
Mn1 - N1	2.3655(13)	2.33(4)	2.2	-
Mn2 - N2	2.3125(13)	2.32(4)	2.3	-

Electrospray ionisation mass-spectrometry (ESI-MS) analysis of **7** revealed a major peak at $m/z = 805.1790$ that corresponded well to the $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2]^+$ ion expected at $m/z = 805.1743$ (A6.2). Comparison of the infrared spectroscopy (IR) spectra of TPDP and complex **7** revealed noticeable differences that indicated complexation has occurred (Figure 6.5). The broad O-H stretch at 3300 cm^{-1} in the TPDP spectra assigned to the hydroxyl group on the ligand backbone was no longer present in the spectrum of **7** (A6.3). Furthermore, in the IR spectrum of complex **7** the symmetric and asymmetric stretches of the carboxylate groups of the bridging benzoates were visible at 1567 cm^{-1} and 1381 cm^{-1} respectively. The stretch originating from the counterion, sodium tetraphenylborate, was also visible in the spectra of **7** at 707 cm^{-1} .

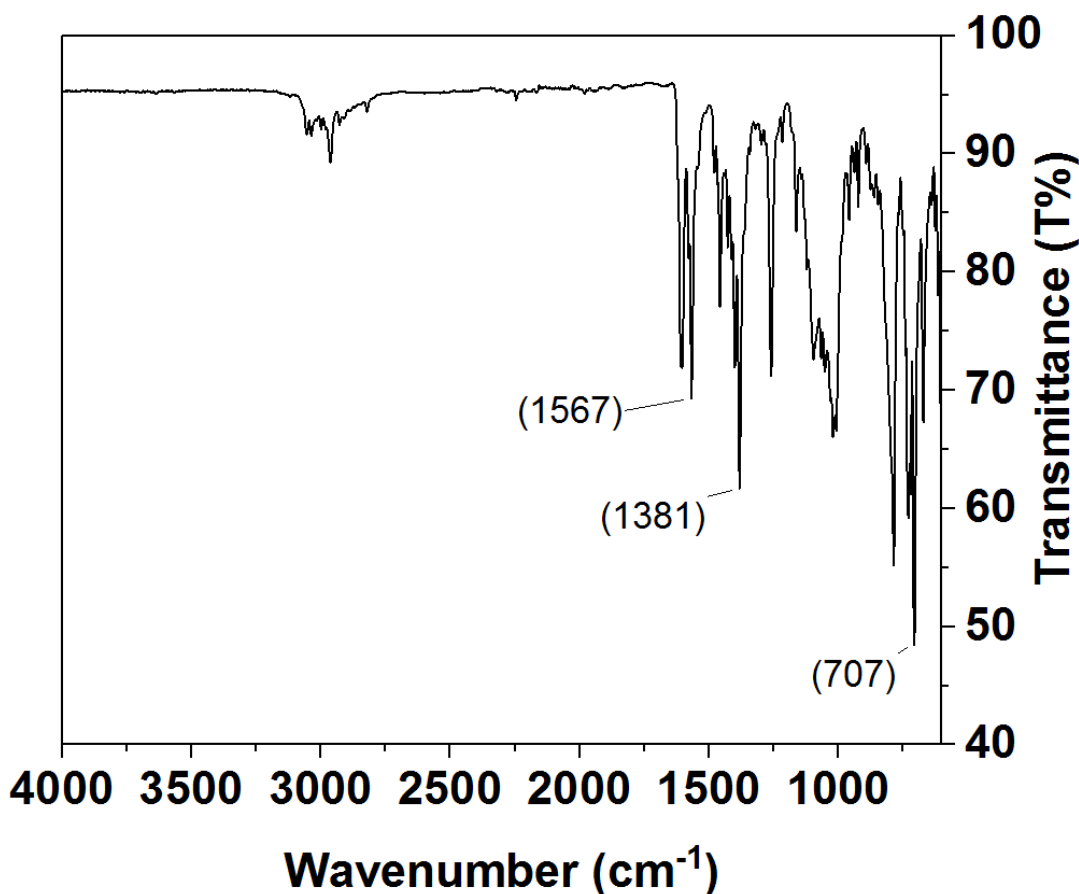


Figure 6.5: IR spectrum of $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$ (**7**)

6.3 Generation of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -Peroxide

A 1.5 mM solution of **7** in 1:9 MeCN/ tetrahydrofuran (THF) was cooled to -90°C . The UV-vis spectra of **7** displayed a saturated absorbance between $\lambda_{\text{max}} = 200$ and 280 nm and a broad shoulder at $\lambda_{\text{max}} \sim 350$ nm (A6.5). Upon addition of 1 eq. of KO_2 (0.04 M) solubilised with cryptand (0.08 M) there was a change in the electronic absorption features of **7**. Two new bands formed, one at $\lambda_{\text{max}} = 450$ nm and a less intense band at $\lambda_{\text{max}} = 590$ nm and there was a red-shift of the saturated absorbance feature at $\lambda_{\text{max}} \sim 250$ nm, defined as new species **8** (Figure 6.6). Addition of greater than 1 eq. of KO_2 resulted in the formation of side products as evidenced by the reduction in the peak-to-trough separation of the $\lambda_{\text{max}} = 450$ nm peak and an overall less defined feature (A6.6). Generation of **8** using 1 eq. of KO_2 resulted in a metastable species with a half-life of $t_{1/2} = 2015$ s.

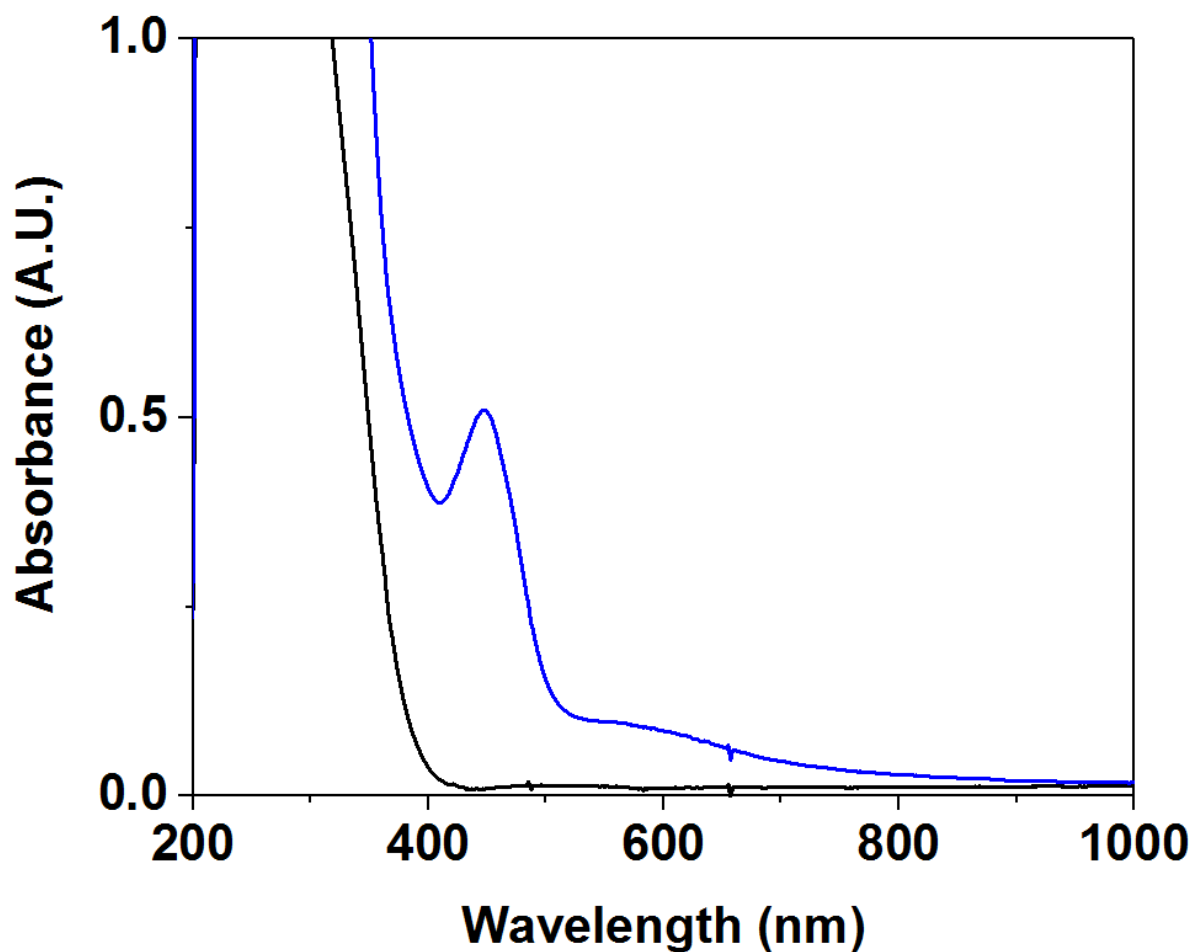


Figure 6.6: UV-Vis spectra of the reaction of 1.5 mM **7** (black trace) + 1 eq. KO_2 to form **8** (blue trace) in 1:9 MeCN/THF at -90°C .

Interestingly, the features of new species **8** were remarkably similar to those of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species previously characterised by the McDonald group.^{4,5} The first $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide reported by the McDonald group, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$, displayed almost identical peaks at $\lambda_{\text{max}} = 460\text{ nm}$ and 610 nm . The similarities' do not end there, as McDonald published another $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^+$, supported by a polypyridine ligand system that also presented an intense peak at $\lambda_{\text{max}} = 440\text{ nm}$ and a less intense feature at $\lambda_{\text{max}} = 590\text{ nm}$.⁵ Due to the similarities with the two previously published species, we tentatively assigned new species **8**, as a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide. In analogy to previously studied Mn^{III} -peroxides, it was postulated that the origin

of the intense feature of **8** at $\lambda_{\text{max}} = 460$ nm was peroxide to Mn^{III} charge transfer and the less intense $\lambda_{\text{max}} = 610$ nm feature was derived from *d-d* transitions.⁴

Electron paramagnetic resonance (EPR) spectroscopy measurements of **8** at 2 K revealed a 22-line signal centred at $g \sim 2$ (Figure 6.7). EPR spectra of Mn_2 complexes with >20 lines is indicative of the formation of a mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species.¹⁵ The large number of lines can be explained due to the hyperfine interaction of the two ^{55}Mn atoms.¹⁶ In a system with two ^{55}Mn equivalent centres and a $S = \frac{1}{2}$ ground state 11-lines would be expected in an isotropic environment; $(2nI + 1)$ where n = number of metal centres and ^{55}Mn $I = 5/2$. In a system where the two Mn atoms are non-equivalent, such as a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complex, this results in a 36 line isotropic signal $((2nI_1 + 1)(2mI_2 + 1) = 6*6 = 36)$.¹⁷ Despite the large number of predicted hyperfine lines, in practice for $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ systems typically between 20-30 are observed due to overlap of the lines. Curiously, the EPR of **8** looked almost identical to that of the first $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide $[\text{Mn}_2(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ reported by McDonald and co-workers, despite having different supporting ligands, solvent systems, and counterions.¹⁸ Due to the characteristic features of the EPR spectra of **8** and its similarity with a previously reported $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide we assigned **8** to be a mixed-valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide.

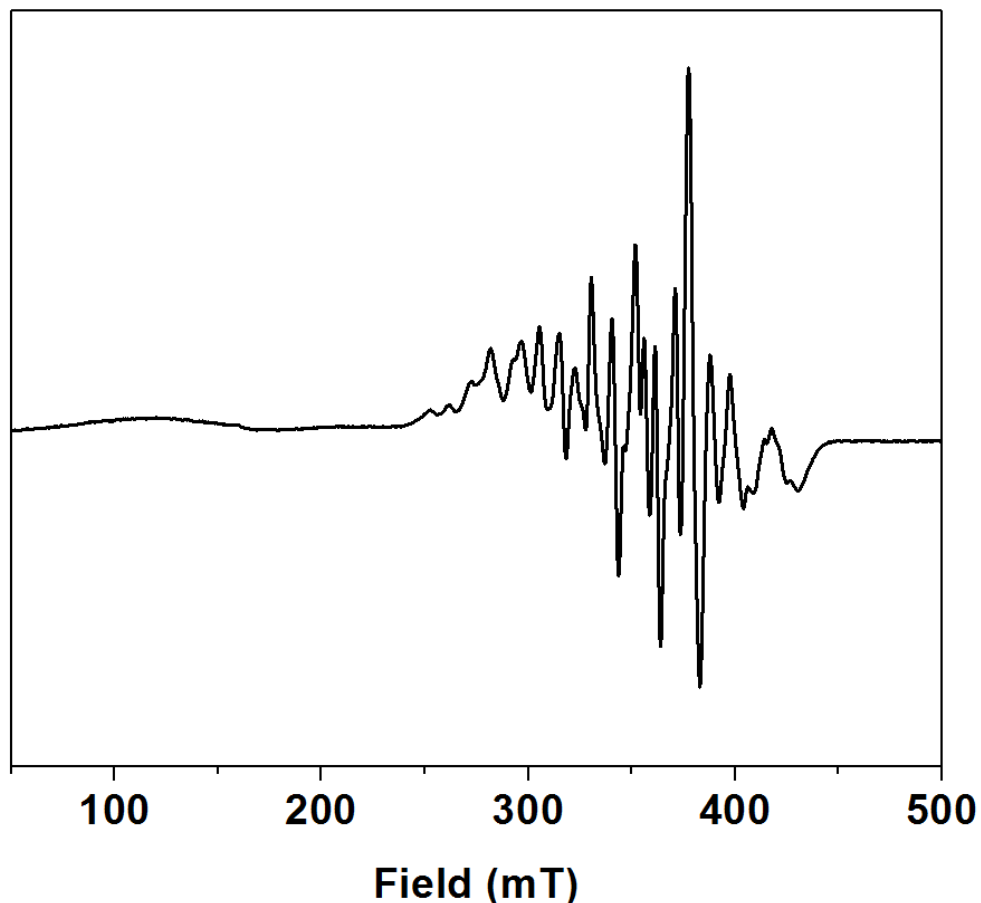


Figure 6.7: Perpendicular X-band EPR spectrum of **8** recorded at 2 K obtained from **7** (1.5 mM) + 1 eq. KO_2 in 1:9 MeCN/THF 9.64 Ghz, 0.2 mW microwave power.

ESI-MS analysis of a just-thawed methanol solution of **8** showed a peak at $m/z = 296.561$ that was consistent with the simulated peak of $m/z = 296.5526$ for the dictation species $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{TPDP})]^{2+}$ (Figure 6.8). The spacing between the peaks was 0.5 units and further agreed with the assignment of a dication species. In the observed dication, both of the bridging benzoate ligands were absent. A similar observation was reported by the McDonald group during ESI-MS experiments with the two studied $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides, both of which did not contain the bridging acetate ligands by ESI-MS that they had been prepared with.^{5,18}

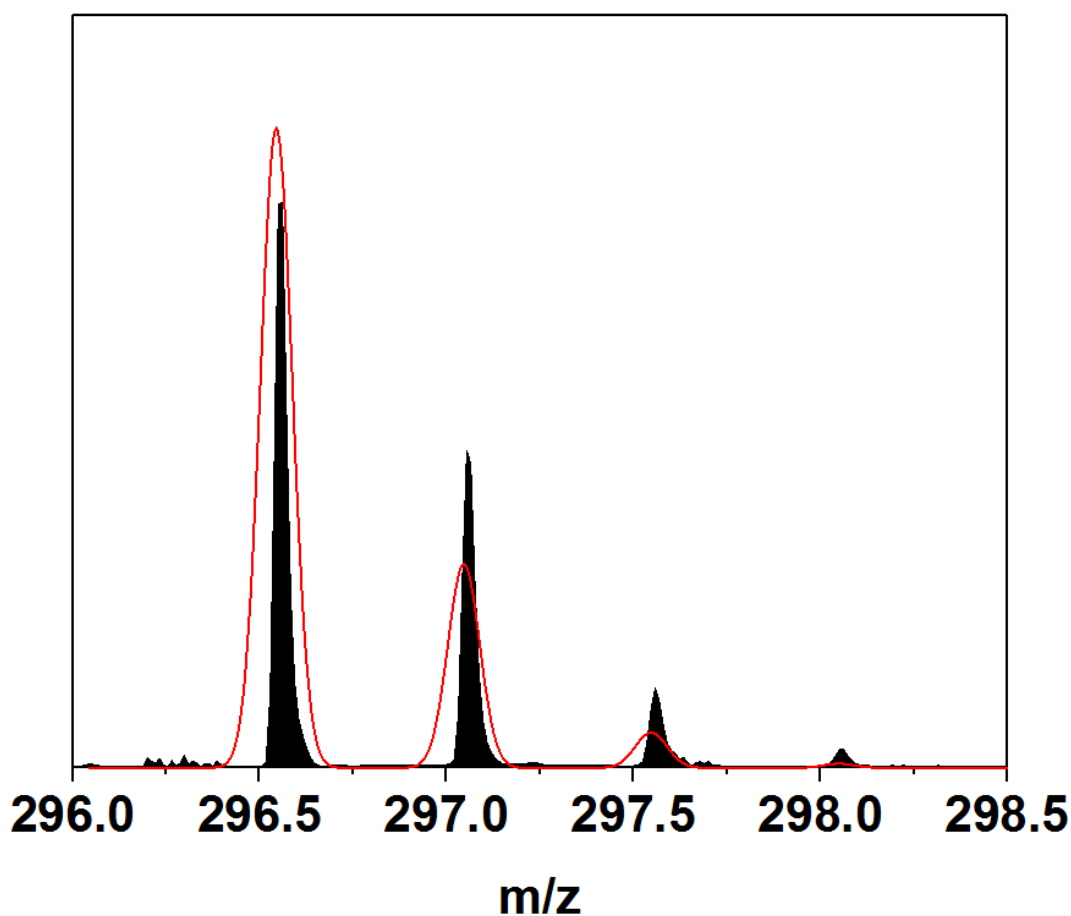


Figure 6.8: ESI-MS spectra of **8** prepared using $K^{16}O_2$ (black trace) and the simulated spectrum (red trace)

Metal-peroxide complexes display characteristic O-O stretches that can be observed using Raman vibrational spectroscopy. A frozen sample of **8** was prepared from 1.5 mM **7** + 1 eq. KO_2 in 1:9 MeCN/THF and excited at 457 and 561 nm. The THF solvent was found to “mask” key regions of the spectra where metal-peroxide complexes are expected to resonant and no characteristic peaks could be observed (A6.8 and A6.9). Hence, alternative solvent systems were explored. Species **8** was generated using acetone as a solvent at -40°C and resulted in identical UV-vis features at $\lambda_{\text{max}} = 450$ nm and 590 nm compared when **8** was generated using the 1:9 MeCN/THF solvent system (Figure 6.9, inset). Therefore, we were confident the same complex, **8**, was generated in solution. A sample of **8** in acetone was excited at 457 and 561 nm. Using the 457 nm laser line, a small peak at 659 cm^{-1} was

observed in sample **8** that was not present in the spectrum of the low-valent complex **7**. This feature at 659 cm^{-1} was further enhanced when the laser line was changed to 561 nm (Figure 6.9). Unfortunately, no other peaks unique to complex **8** were observed in the Raman spectra measured using acetone as a solvent system (A6.9 – A6.11).

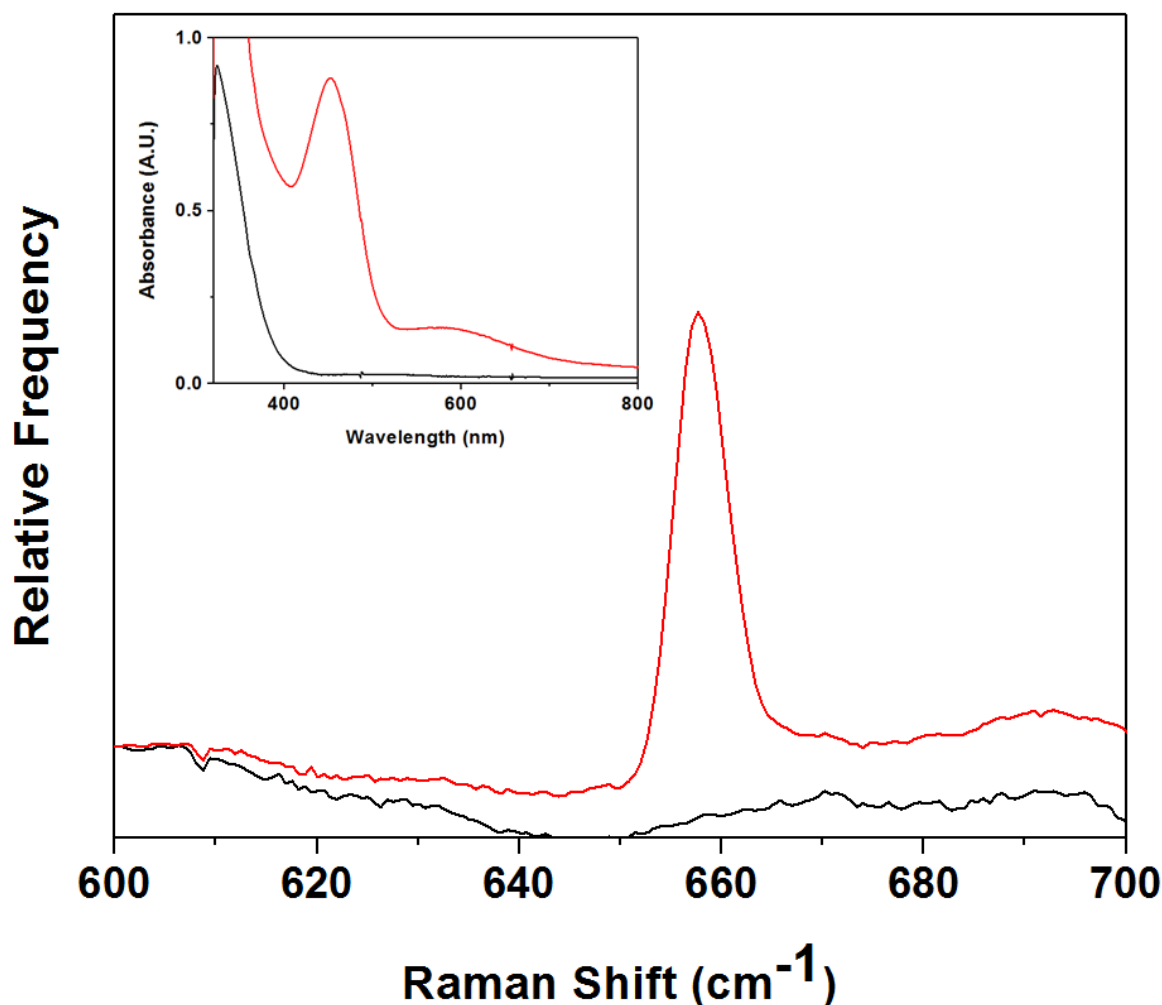


Figure 6.9: Resonance Raman spectra of **7** (black trace) and **8** (red trace) in acetone excited at 561 nm . Inset: UV-vis spectra of the formation of **8** from **7** ($1.5\text{ mM} + 2\text{ eq. KO}_2$) in acetone at $-40\text{ }^\circ\text{C}$.

Raman spectroscopy studies of metal-peroxide complexes have been reported for a variety of metals including Mn, Fe, and Co. Typically, the $\nu_{\text{O-O}}$ stretches of these complexes are observed between 800 and 900 cm^{-1} (Table 6.3). For example, the Mn^{III} -peroxide complex $[\text{Mn}^{\text{III}}(\text{O}_2)(\text{H}_3\text{bupa})]$ exhibited an $\nu_{\text{O-O}}$ stretch at 885 cm^{-1} that shifted by 48 cm^{-1} upon

substitution with $^{18}\text{O}_2$.¹⁹ The $\nu_{\text{Mn-O}}$ stretch in metal peroxides can also be observed using Raman spectroscopy. Kovacs *et al.* reported the $\nu_{\text{Mn-O}}$ stretch of a dinuclear Mn^{III}_2 - peroxide species at 611 cm^{-1} and a shift of 25 cm^{-1} upon labelling with $^{18}\text{O}_2$. This was the only Mn complex for which the $\nu_{\text{Mn-O}}$ stretch has been reported. However, other metal containing peroxide complexes, for example, Fe and Co, have also reported a $\nu_{\text{M-O}}$ that fall between $470 - 611\text{ cm}^{-1}$ (Table 6.3). Due to solvent absorption in the $800 - 900\text{ cm}^{-1}$ region of the spectrum, we were not able to observe a $\nu_{\text{O-O}}$ for complex **8**. However, we observed a stretch at 659 cm^{-1} that may be the $\nu_{\text{M-O}}$. Although the resonance fell higher than the previously recorded $\nu_{\text{M-O}}$ for a Mn-peroxide complex, none of these species were $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides like **8**. Furthermore, wide range of Raman shifts for $\nu_{\text{M-O}}$ can exist even within similar complexes. For example, within the related Fe-peroxide tmp complexes supported by porphyrin ligands shown in Table 6.3, a difference of over 100 cm^{-1} was seen between the $\nu_{\text{M-O}}$ stretches. Therefore, we postulate that the peak observed at 659 cm^{-1} for complex **8** may be a $\nu_{\text{Mn-O}}$ stretch of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species.

Table 6.3: Reported resonance Raman stretches associated with metal-peroxide complexes reported in the literature.

Complex	(ν_{O-O} , $\Delta^{18}O$ /cm ⁻¹) 1)	(ν_{M-O} , $\Delta^{18}O$ /cm ⁻¹) 1)	Ref
{[Mn ^{III} (S ^{Me2} N ₄ (6-Me-DPEN))] ₂ (trans- μ -1,2-O ₂)} ²⁺	819, 47	611, 25	20
[Mn ^{III} (O ₂)(3,5-iPr ₂ pzH)(HB(3,5-iPr ₂ pz) ₃)]	892, 51	-	21
[Mn ^{III} (O ₂)(H ₃ bupa)] ⁻	885, 48	-	19
[(tmp)Fe ^{III} (O ₂)]	809, 45	470, 19	22
[(tmpIm)Fe ^{III} (O ₂)]	807, 49	475, 20	22
[(tmpImXan)Fe ^{III} (O ₂)]	808, 38	585, 25	23
[Co ^{III} (13-TMC)(O ₂)] ⁺	902, 56	542, 22	24

Mn K-edge X-ray absorption near edge spectroscopy (XANES) was performed on frozen samples of **7** and **8** in order to accurately understand how the Mn oxidation states and local geometries were affected during reaction with superoxide (Figure 6.10). The low-valent complex **7** showed a rising edge with the first inflection at 6547.2 eV. **7** also displayed a pre-edge peak that was modelled using a 50:50 pseudo-Voigt function (A6.12). Modelling determined the pre-edge peak was centred at 6540.3 eV had an area of 15.2 units. For complex **8**, the first inflection point of the rising edge was determined to be 6547.2 eV. **8** also displayed a pre-edge peak that could be modelled using a 50:50 pseudo-Voigt function (A6.13) and was determined to be centred at 6540.8 eV with an area of 8.8 units.

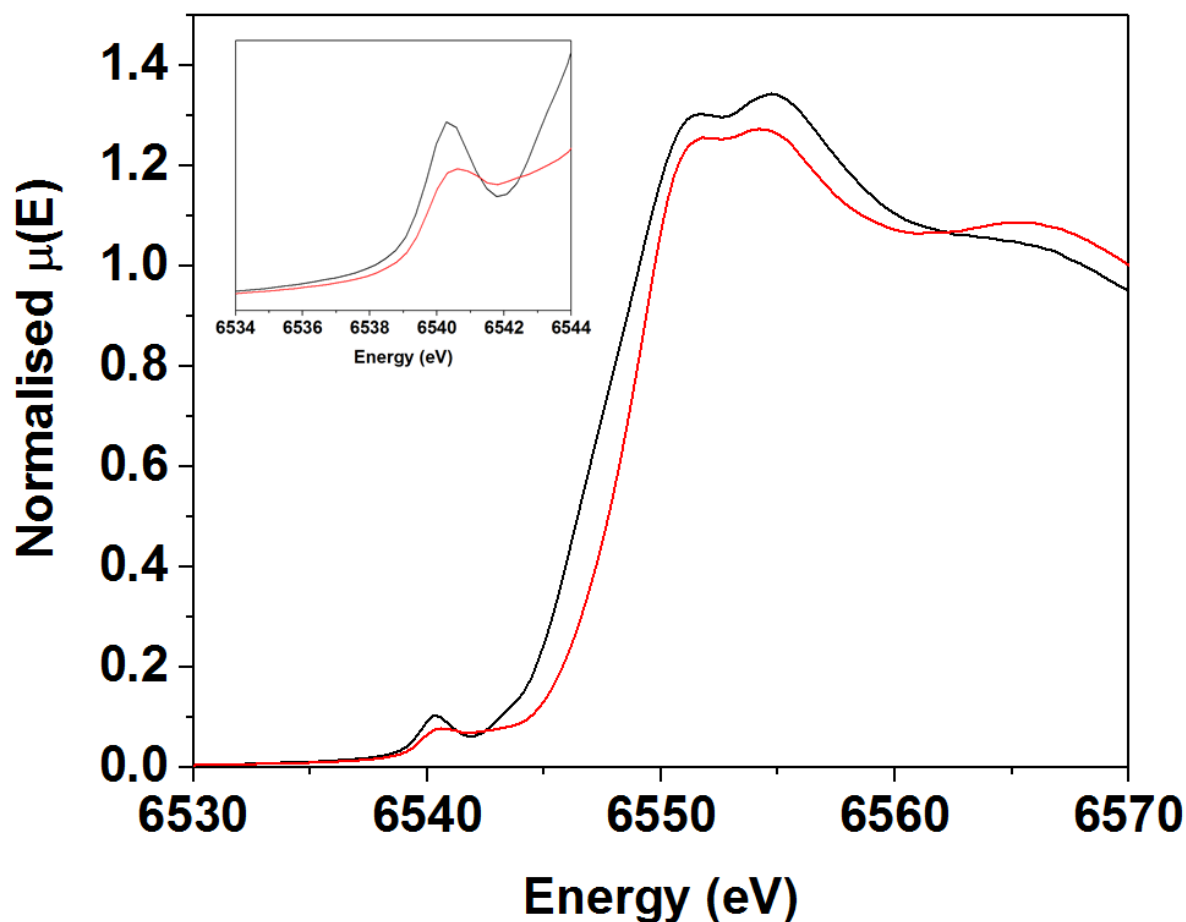


Figure 6.10: Normalised XANES spectra of **7** (black trace) and **8** (red trace). Inset: Expansion of pre-edge region.

Upon conversion of **7** to **8** there was an edge energy shift of 2.1 eV. Typically, an increase of one in the formal oxidation of Mn was associated with an edge energy shift of 2-4 eV.^{25,26} A shift of 2.1 eV was consistent with the +1 increase in oxidation state on the conversion of a Mn^{II}_2 species (**7**) to the mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species (**8**) and agreed well with the EPR data of **8**. The rising edge of **7** also exhibited a shoulder at ~6552 eV that was characteristic of the presence of Mn^{II}_2 .²⁷ Noticeably, this shoulder decreased in intensity in the spectrum of **8**, and suggested that less Mn^{II}_2 was present in the sample of **8**. This was further supportive of the conversion of low-valent Mn^{II}_2 (**7**) to a high-valent species. The pre-edge, i.e. the peak before the main edge, can also provide insight into the local structures of the Mn centres.²⁵ The pre-edge was associated with crystal field transition from

the core 1s orbitals to the 3d levels, which can gain some 4p character depending on the deviation from centrosymmetry.²⁸ This resulted in complexes with more distorted geometries displaying larger pre-edge areas than those with less distorted ones. The pre-edge areas of **7** and **8** were calculated to be 15.2 and 8.8 units respectively. Therefore, upon conversion of complex **7** to **8** there was a decrease in the pre-edge area of 6.4 units. This implied that the Mn centres in **8** had a more centrosymmetric geometry in comparison to those of **7**. One possibility is that the benzoic acid moieties of **7** were leading to larger distortions around the Mn^{II} centres and upon conversion to **8** there was a loss of these bridging ligands and resulted in a more centrosymmetric environment. This would agree with the mass spectrometry data of **8** that showed loss of the bridging benzoate ligands upon reaction with superoxide to form a Mn^{II}Mn^{III}-peroxide species. The centre of the pre-edge peaks of **7** and **8** agreed well with values at 6540.3 and 6540.8 eV respectively. This was consistent with an assignment of a Mn^{II}Mn^{III} oxidation states as appreciable changes in the pre-edge of Mn complexes do not occur until the Mn^{IV} oxidation state.²⁵ Therefore, with the combination of UV-vis, EPR, and X-ray absorption spectroscopies and mass spectrometry we assign the conversion of a Mn^{II}₂ complex (**7**) to a new Mn^{II}Mn^{III}-peroxide (**8**) upon reaction with 1 eq. superoxide (Figure 6.11).

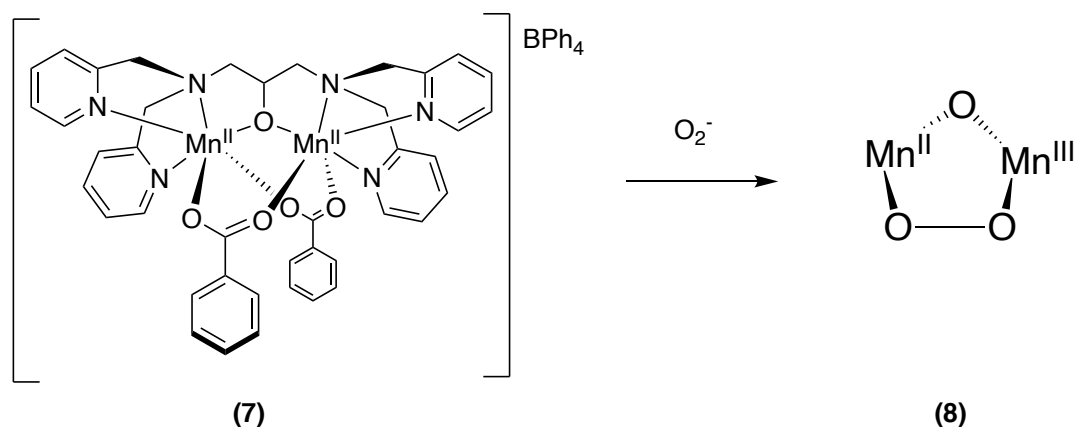


Figure 6.11: Generation of **8** via addition of 1 eq. of O_2^- to **7**.

As we have established the formation of a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species (**8**), we were interested in probing the reactivity of **8**. The previously studied $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complexes studied by the McDonald group ($[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$) were determined to be poor oxidants, incapable of reacting with substrates with weak O-H bonds such as 2,2,6,6-tetramethylpiperidin-1-ol (TEMPO-H) with an O-H bond dissociation energy (BDE) of 70.6 kcal mol⁻¹.^{4,5,29} Therefore, we were interested in seeing if the new ligand system of **8** had afforded any increased reactivity for the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide.

Monitoring by UV-vis spectroscopy, 30 eq. of TEMPO-H was added to **8** at -90 °C (A6.14). Addition of TEMPO-H to **8** resulted in a slight decrease in the intensity of absorption at the $\lambda_{\text{max}} = 450$ nm feature owing to the dilution effect of addition of the substrate. A reduction of the peak-to-trough ratio was observed for the $\lambda_{\text{max}} = 450$ nm and a slight broadening of the feature at $\lambda_{\text{max}} = 590$ nm was also observed. However, these changes of the UV-vis features of **8** are also observed during the decay of the species when no substrate was present. Therefore, we postulated that **8** was not capable of oxidising O-H bonds as no changes of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide spectra of **8** were observed upon addition of TEMPO-H. EPR analysis of the post-reaction mixture confirmed this as the TEMPO $\cdot$ radical was formed in a yield of <10% (A6.15). Moreover, addition of 5 eq. of ferrocene to complex **8** at -90 °C was monitored via UV-vis spectroscopy and also yielded no change in the electronic absorption features of **8** and no new feature at $\lambda_{\text{nm}} = 620$ nm, indicative of formation of the ferrocenium cation, appeared (A6.16).⁴ **8** was therefore unreactive in the presence of O-H bonds and reducing agents and appeared to be a poor electrophile, similar to the previously studied $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides. The lack of reactivity observed in all of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides appeared to be intrinsic to this type of complex. This perhaps

explained the need of a proton in the catalytic cycle of class Ib RNRs, to convert the generated $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide to a more reactive species for the oxidation of the tyrosine residue.

In conclusion, a new Mn^{II}_2 complex (**7**) was found to react with superoxide (O_2^-) to generate a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species (**8**) via low-temperature UV-vis spectroscopy. Further structural analysis of **8** via EPR and X-ray absorption spectroscopies and mass spectrometry lead to the assignment of **8** to be a new mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species. To the best of our knowledge, **8** was only the third $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide to have been characterised.^{4,5} Moreover, **8**, like the two other $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides, was generated following reaction of a Mn^{II}_2 complex with superoxide. This provided further weight to the argument that a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide was the unknown intermediary species formed in the cycle of Ib RNRs as it appeared to be thermodynamically favoured by the mimics of the enzymes active site.

6.4 Comparison of $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -Peroxides

A new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**8**) was synthesised and we were interested in comparing the spectroscopic properties of the new complex to the two previously characterised $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides; $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ (**2**). At first glance, the electronic absorption features of the three different metal peroxides appeared very similar (Figure 6.12). All three displayed a feature between $\lambda_{\text{max}} = 440 - 450$ nm and a less intense feature $\lambda_{\text{max}} = 570 - 590$ nm. These spectroscopic features appeared to be a fingerprint for this class of complex. Upon closer inspection, some differences were observed between the three. Both **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ (blue and purple trace respectively, Figure 6.12) had electronic absorption features centred at $\lambda_{\text{nm}} = 450$ and 590 nm. While in comparison, the respective electronic features of **2** were shifted to the

slightly higher energy wavelengths of $\lambda_{\text{nm}} = 440$ and 570 nm (red trace, Figure 6.12). This was indicative that the electronic environment of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide core of **2** was different to that of **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$.

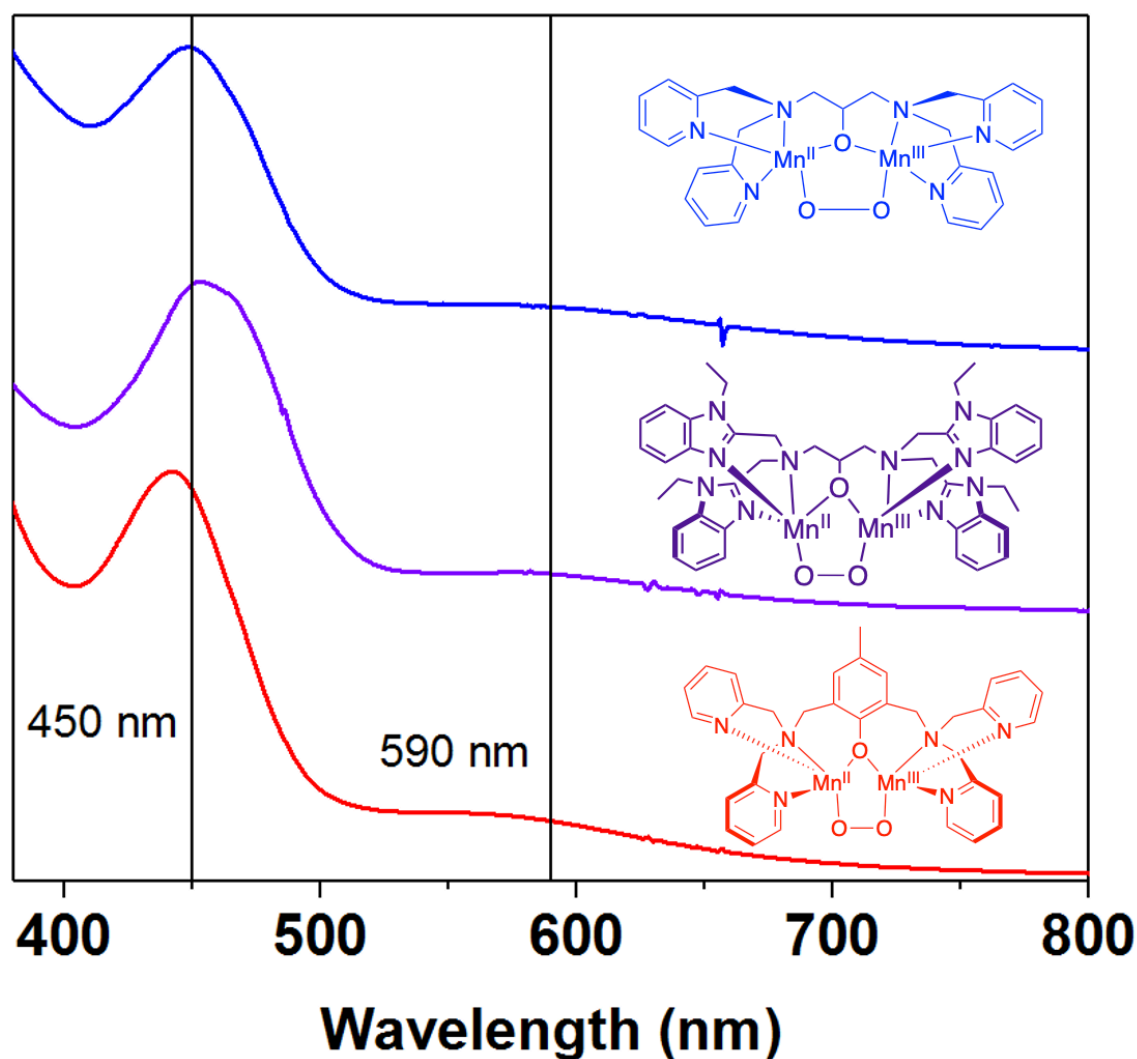


Figure 6.12: Electronic absorption spectra of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{TPDP})]$ (**8**, generated from $1.5 \text{ mM } \mathbf{7} + 1 \text{ eq. } \text{KO}_2$ in $1:9 \text{ MeCN/THF}$ at -90°C , blue trace), $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ (**2**, generated from $1.5 \text{ mM } \mathbf{1} + 1 \text{ eq. } \text{KO}_2$ in $1:9 \text{ MeCN/THF}$ at -90°C , red trace), and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ (generated from $1.5 \text{ mM } [\text{Mn}^{\text{II}}_2(\text{OAc})(\text{N-Et-HPTB})](\text{ClO}_4) + 1.25 \text{ eq. } \text{KO}_2$ in MeCN at -40°C , purple trace).

The EPR spectra of the three $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ peroxide complexes can also be compared. At 2K , all three complexes displayed multi-line signals centred at $g = 2$ with a wide spectral range of $\sim 200 \text{ mT}$ (Figure 6.13). Once again, complexes **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$

displayed very similar, almost identical, spectra. One of the only differences was that $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ displayed a small peak at that indicated 100 mT the presence of spin states $S > \frac{1}{2}$ where **8** does not. The EPR spectrum of **2**, however, was very different to that of **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$. The spectrum of **2** appeared to be more uniform compared to the other two complexes, a slightly wider spectral width, and a more intense signal centred at ~ 150 mT, assigned to the presence of high spin states. The disparity between spectra once again illustrated the differences between the electronic structure of **2** compared to **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$.

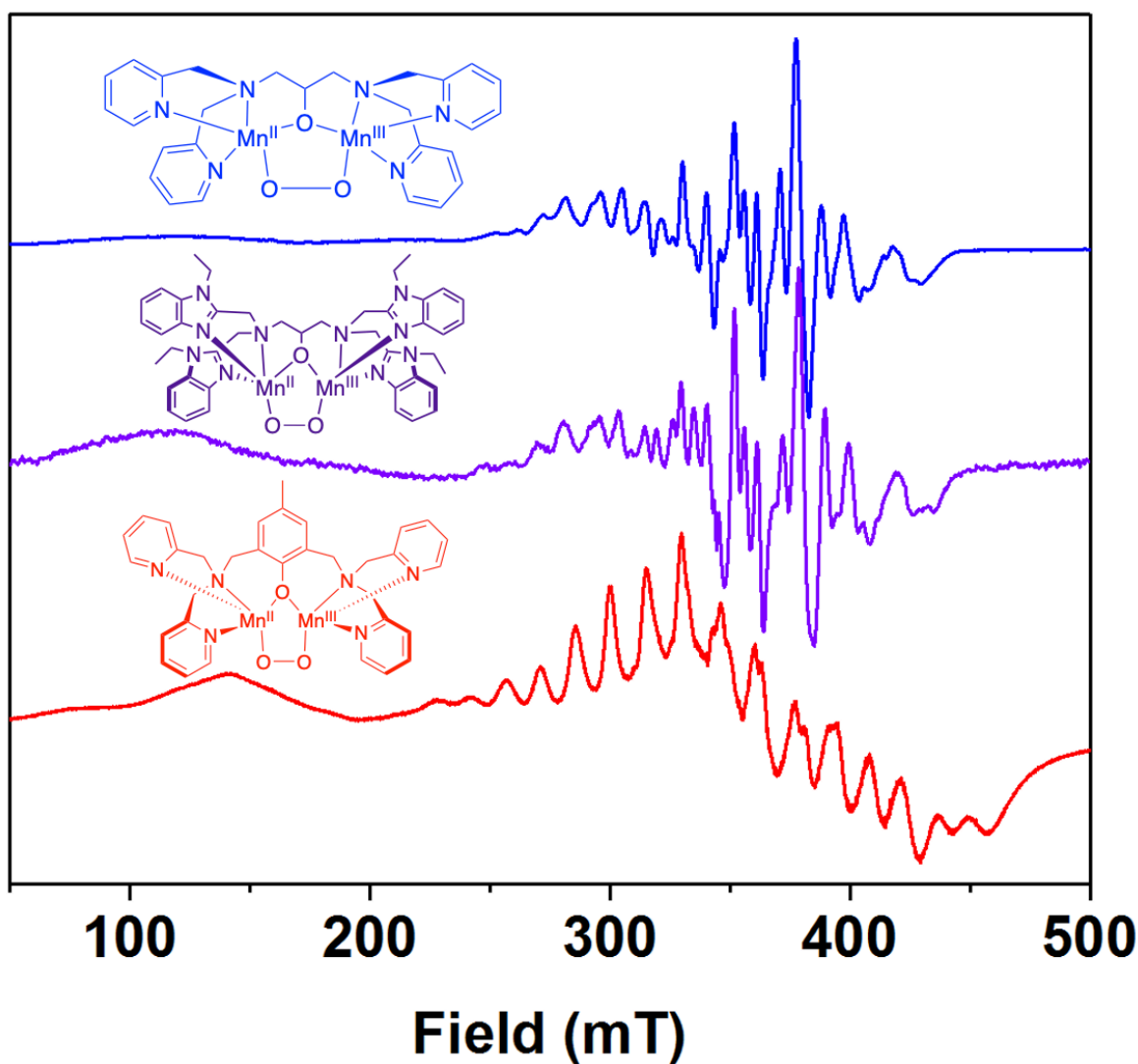


Figure 6.13; Blue trace; Perpendicular X-Band EPR spectrum of **8** measured at 2 K (9.65 GHz, 0.2 mW microwave power). Purple trace; Perpendicular X-Band EPR spectrum of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ measured at 2 K (9.65

GHz, 0.2 mW microwave power). Red trace: Perpendicular X-Band spectrum of $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{BPMP})]^{2+}$ measured at 2 K (9.65 GHz, 0.2 mW microwave power).

Finally, we can also compare the reactivity of the three complexes. Complex **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ shared very similar UV-vis and EPR spectra and both complexes were determined to be quite unreactive.¹⁸ Complex **2**, on the other hand, although unreactive with weak O-H bonds, was found to be capable of the deformylation of aldehyde substrates. This was in stark contrast to the lack of reactivity displayed by **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$.

Therefore, there appeared to be a trend among these three $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complexes, where **2** was an outlier. Comparison of the structures of the three was quite interesting because **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ had different ligands, solvent systems, counterions, and were generated at different temperatures but displayed very similar spectroscopic properties and reactivity. In contrast, complexes **2** and **8** both contained similar chelating polypyridyl ligands, use the same solvent system, and are generated at the same temperature. Yet, both **2** and **8** displayed differing spectroscopic properties and reactivity. The one feature that was present in complex **2** and not observed in complexes **8** and $[\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}(\text{O}_2)(\text{N-Et-HPTB})]^{2+}$ was the phenol moiety on the backbone of the BPMP ligand of **2**. We postulated that the difference in UV-vis and EPR features and increase in reactivity observed in **2** was due to the presence of the aromatic phenol moiety in its' ligand backbone. The phenol in the backbone of the BPMP ligand was directly bonded to the Mn centres of the peroxide in **2** and could have impacted the electronic environment of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide core. We postulated that the phenol exerted an electron-withdrawing effect upon the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide core of **2**, effecting the spectroscopic properties of **2** and also in turn increased the reactivity of the complex.

6.5 Reaction of **8** with an Acid

Previously, we had success with activation of a similar $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide to form a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex using *para*-toluenesulfonic acid (pTsOH) as the proton source (Chapter 3). Hence, we were interested in probing the reactivity of **8** towards pTsOH. To a 1.5 mM solution of **8** in 1:9 MeCN/THF at $-90\text{ }^{\circ}\text{C}$, 1 eq. of pTsOH was added and the reaction was monitored via UV-vis spectroscopy. A resulting slow reaction was observed that took 2000 seconds to complete, indicative of the formation of a new species (**9**) (Figure 6.14). **9** displayed a long, broad electronic absorption spectra that tailed into the near-IR region. It was observed that addition of just one equivalent of pTsOH did not completely consume **8** as the feature at $\lambda_{\text{max}} = 450\text{ nm}$ indicated the presence of unreacted $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide. Addition of 2 eq. pTsOH appeared to react with all of **8**, as evidenced by the complete disappearance of the peak at $\lambda_{\text{max}} = 450\text{ nm}$, and revealed **9** has a broad, featureless UV-vis spectrum that tailed into the near IR region (Figure 6.14).

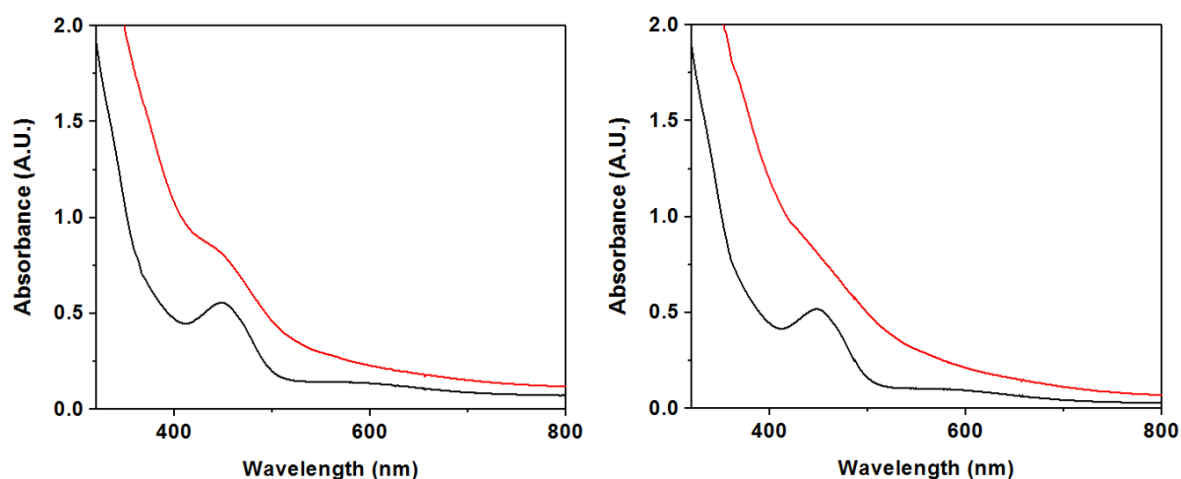


Figure 6.14: UV-Vis features of the reaction of **7** + 1 eq. pTsOH (left) and **7** + 2 eq. pTsOH (right) in 1:9 MeCN/THF at $-90\text{ }^{\circ}\text{C}$.

In order to probe the identity of this new species, EPR was performed at 2 K on a frozen samples of **8** + 1 eq. pTsOH and **8** + 2 eq. pTsOH (**9**) (Figure 6.15). The 2 K EPR spectra

of the post-reaction mixture of **8** + 1 eq. pTsOH revealed two new signals. One broad signal centred at $g = 6.05$ and a 23-line signal centred at $g \approx 2$. The broad signal at a high g -tensor has been observed previously for $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes and was assigned as transitions from $S = 3/2$.^{4,16,30} Furthermore, the multiline signal at $g \approx 2$ was also indicative of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species, due to high number of lines and broad range, however, assignment of this new species was limited as unreacted $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide (**8**) was present in solution that could be clearly seen in the 300 – 400 mT range.

In contrast, the EPR spectra of **9** (**8** + 2 eq. of pTsOH) showed no remaining $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, verifying that 2 eq. of the acid were required in order to completely consume **8**. In the EPR spectrum of **9**, the broad signal associated with $S = 3/2$ spin states at $g = 6.05$ has increased in intensity. More importantly, a 21-line signal centred at $g \approx 2$ could be more clearly seen. $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes are known for displaying a large number of lines in the EPR spectrum, typically between 20 and 30, originating from a hyperfine interaction between the non-equivalent Mn centres.^{17,31,32} This signal displayed quite a large spectral width of approximately 200 mT. This was further emblematic of a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ system and also stemmed from their irregular hyperfine pattern.^{17,31} For example, the complex $[\text{Mn}^{\text{II}}(\text{OH})\text{Mn}^{\text{III}}]^+$ reported by Borovik and co-workers also displayed a multiline line signal centred at $g = 2$ with a range of ~ 200 mT.³³ In contrast, $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes will display much narrower ranges.^{34,35} Furthermore, the EPR spectra of **9** was not resolved and broadened significantly when measured at temperatures > 10 K (A6.17). This type of EPR temperature dependence was associated with $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complexes, which must be measured at temperatures < 15 K in order to be completely resolved.^{5,16,17,31,32,36} This further supported that **9** contained a mixed valent $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ oxidation state. Crucially, **9**'s new signal at $g \approx 2$ displayed a different number of lines and shape compared to the similar

signal of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide of **8**, implying that **9** was a different $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ system than **8**.

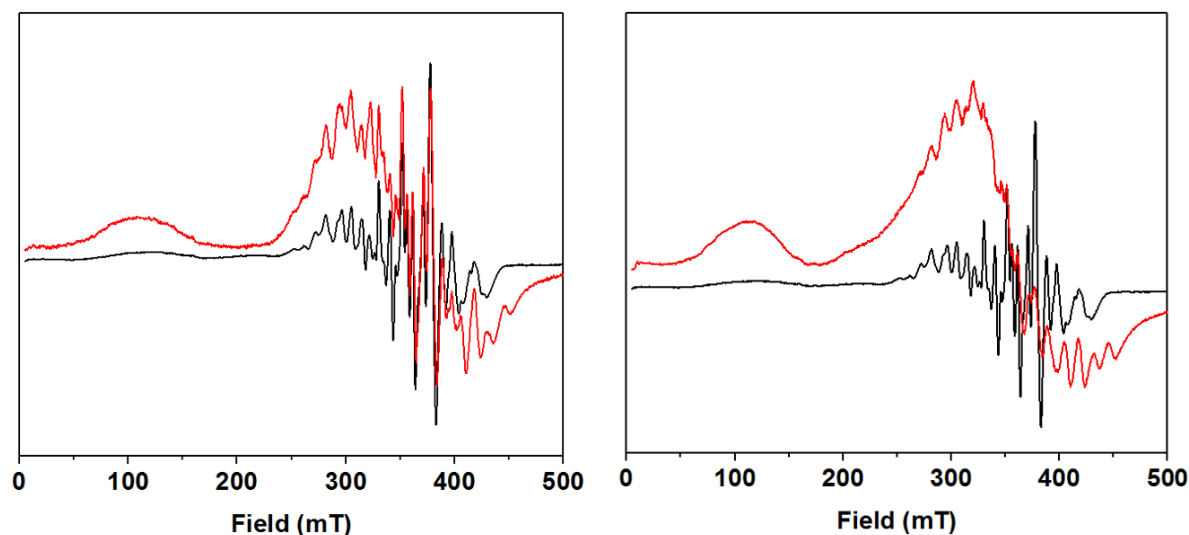


Figure 6.15: Perpendicular X-Band EPR spectrum of **8** + 1 eq. pTsOH (left) and **8** + 2 eq. pTsOH (right) measured at 2 K (9.65 GHz, 0.2 mW microwave power).

To further analyse the Mn oxidation states and their local geometries, Mn K-edge XANES was performed on **9** (Figure 6.16). **9** displayed a rising edge with the first inflection at 6549.4 eV and a pre-edge peak modelled using a 50:50 pseudo-Voigt function (A6.18). Modelling determined the pre-edge peak was centred at 6540.4 eV an area of 3.9 units. Initially, the XANES spectra of **8** and **9** appeared very similar. Upon conversion of **8** to **9** there was a corresponding edge energy shift of 0.1 eV (Table 6.4). This shift in edge energy was negligible, as Mn centres typically displayed an edge energy shift between 2- 4 eV when an oxidation number increases by +1.²⁵ This demonstrated that **8** and **9** shared the same Mn oxidation states. This was consistent with EPR experiments that indicated both **8** and **9** possessed $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ centres. Another similarity between the **8** and **9** was the pre-edge peaks of 6540.8 and 6540.6 eV respectively. The agreement of these peaks further affirmed that both complexes have identical oxidation states. One difference between the

XANES spectra of these complexes was that relative to the spectrum of **8**, complex **9** showed a decrease in the shoulder at ~6552 eV. This shoulder at ~6552 eV in Mn-K-edge spectra has been observed previously in the study of Id RNRs and assigned to Mn^{II}_2 species.²⁷ Therefore we postulated the decrease observed in **9** was indicative of the absence of any residual starting material in the sample.

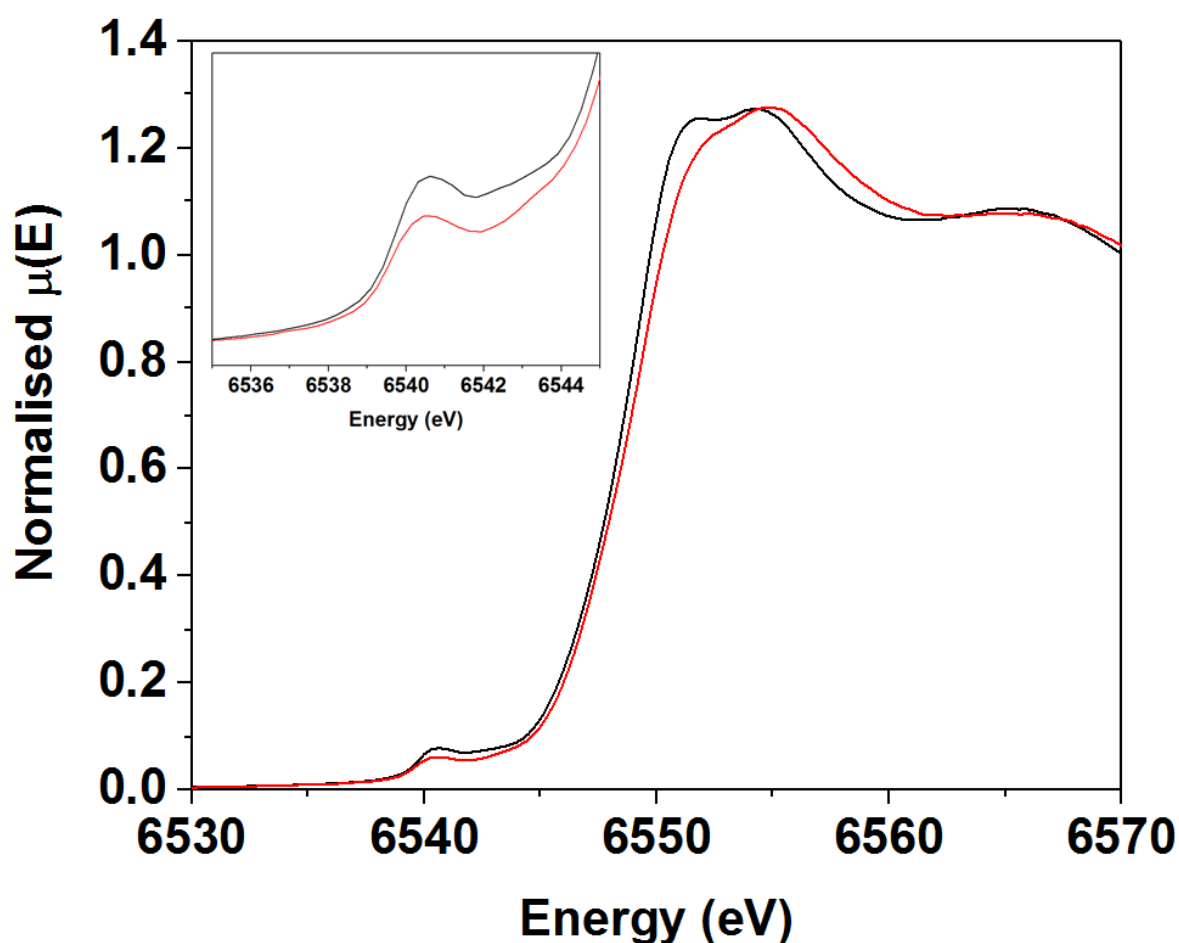


Figure 6.16: Normalised XANES spectra of **7** (black trace) and **8** (red trace). Inset: Expansion of pre-edge region.

Another area of the XANES spectra of **8** and **9** where there was a distinction between the two is the pre-edge areas (Table 6.4). There was a decrease in the pre-edge area of **9** by 4.9 units relative to that of **8**. The pre-edge area was related to the electronic symmetry of the metal centres, with more distorted centres displaying increased edge areas. Therefore, it was postulated that **9** has a more centrosymmetric geometry compared to **8**.²⁸ In 2014, Nam

and co-workers described a Mn^{III} -hydroperoxo species generated from protonation of a Mn^{III} -peroxide that also exhibited a decrease in pre-edge area upon addition of the acid.³⁷ Therefore, it was postulated that **9** contained the same $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ oxidation state as the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **8**, however, due to the characteristic decrease in pre-edge area there appeared to be a protonation of the peroxide bridge to possibly form a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species.

Table 6.4: Comparison of the XANES parameters of complexes **7**, **8**, and **9**.

Complex	Edge Energy (eV)	Pre-Edge Area (units)	Pre-Edge Peak (eV)
7	6547.2 (± 0.1)	15.2 (± 0.1)	6540.3 (± 0.1)
8	6549.3 (± 0.1)	8.8 (± 0.1)	6540.5 (± 0.1)
9	6549.4 (± 0.1)	3.9 (± 0.1)	6540.4 (± 0.1)

Upon addition of 2 eq. of pTsOH to $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species (**8**) there was generation of a new species **9** as evidenced by UV-vis spectroscopy. This new species **9** was investigated by EPR spectroscopy and found to exhibit a 21 line signal centred at $g \approx 2$, characteristic of the formation a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species. XANES studies on new species **9** revealed that there was no significant shift in the Mn K-edge energy of **9** in comparison to **8**, further confirming that both species have the same formal oxidation state values. Interestingly, **9** displayed a decreased pre-edge area compared to **8**, which has been observed before in the generation of Mn^{III} -hydroperoxo species via protonation of a Mn^{III} -peroxide.³⁷ Therefore, we assigned **9** to be a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species formed after reaction of 2 eq. of pTsOH with **8** (Figure 6.17). This represented, to the best of our knowledge, the first case

of a metastable $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species characterised by UV-vis, EPR and X-ray absorption spectroscopies.

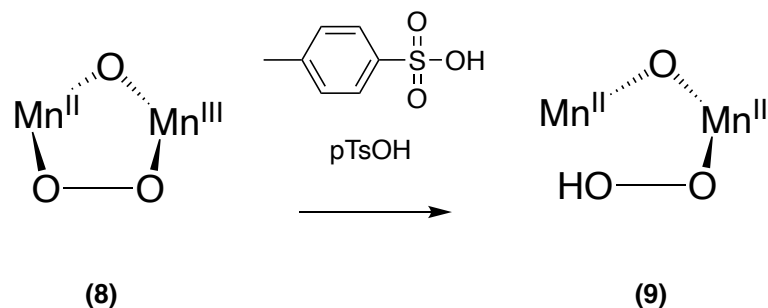


Figure 6.17: The conversion of **8** to **9** upon addition of 2 eq. $p\text{TsOH}$.

The discovery of the structure of **9** to be a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo had interesting implications for the mechanism of Ib RNRs. Previously we found that addition of the acid $p\text{TsOH}$ to the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide supported by a BPMP ligand (Chapter 3) resulted in conversion to a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. However, in this case of TPDP supported $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, addition of the acid resulted in conversion to a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species. This emphasised the role the local environment of the Mn centres plays in determining what species are accessible in the catalytic cycle and underlined the importance in creating small molecule mimics that accurately recreate the ligands of the enzyme.

The $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species (**8**), the precursor to **9**, was found to be unreactive with weak O-H bonds, therefore, we were curious to see if the conversion to a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species (**9**) had any effect on the reactivity of the complex. Addition of 30 eq. of the O-H bond containing substrate TEMPO-H to **9** at $-90\text{ }^{\circ}\text{C}$ was monitored via UV-vis spectroscopy (Figure 6.18). A slow bleaching of the electronic absorption features associated with **9** was observed upon addition of TEMPO-H, indicative that a reaction occurred. EPR spectroscopy was performed on the post-reaction mixture and revealed an intense rhombic signal with $g_x = 2.02$, $g_y = 2.00$, and $g_z = 1.97$. This signal was indicative

of the formation of the 2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO $\cdot$) radical species, and has been observed previously following oxidation of the TEMPO-H substrate.³⁸ The TEMPO $\cdot$ radical yield was calculated to be 40 ± 15 % by EPR considering one-electron oxidation. A control reaction with no **9** present, resulted in a TEMPO $\cdot$ radical yield of <5%, implying that the Mn^{II}Mn^{III}-hydroperoxo species is responsible for the oxidation (A6.19).

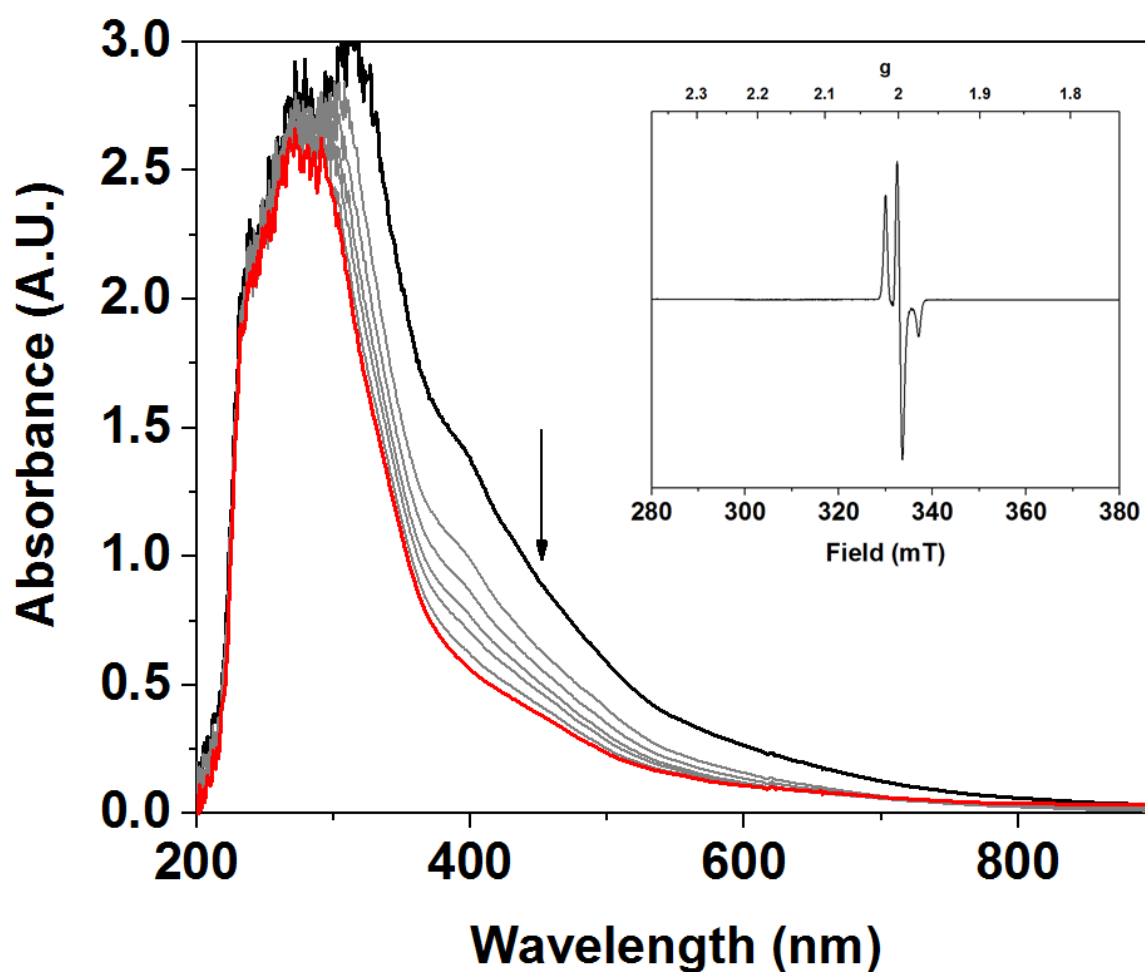


Figure 6.18: UV-Vis spectrum of the reaction of **9** (black trace, generated from 1.5 mM **7** + 1 eq. KO₂ + 2 eq. pTsOH) + 30 eq. of TEMPO-H (red trace) in 1:9 MeCN/THF at -90 °C. Inset: Perpendicular X-Band spectra of the post-reaction mixture of **9** + 30 eq. TEMPO-H (9.34 Ghz, 2.00 mW microwave power)

It was interesting that complex **9** had the capacity to oxidise an O-H bond, while its precursor, **8** showed no reactivity with the same substrate. This once again illustrated the lack of reactivity of Mn^{II}Mn^{III}-peroxide complexes and rationalised why Ib RNRs must

convert to a more reactive species that was capable of oxidising the nearby tyrosine residue. Furthermore, as we were capable of activating the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **8** using a proton source (pTsOH), we postulate that a proton was involved in the process of triggering the conversion of intermediate $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide, as Stubbe *et al.* postulated in their studies of Ib RNRs.³

Evidence is scant of the formation of a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex as the species responsible for oxidation of the tyrosine residue in the catalytic cycle of Ib RNRs. Just one 16-line EPR spectra exists to support its formation.³ Previously, we have generated $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes (Chapters 3 and 4) that were highly capable oxidising species, able to perform one-electron oxidations of *para*-substituted-2,6-di-*tert*-butylphenols. The $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species studied in this chapter on the other hand, displayed a sluggish rate of reaction with the weak O-H bond containing substrate TEMPO-H (A6.20) and displayed moderate yields of the TEMPO $\cdot$ radical product. Therefore, we postulated that a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo was not sufficiently reactive to be the active oxidant species in the enzyme's catalytic cycle and we proposed that a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species was instead employed.

6.6 Conclusions

Synthesis of new dinuclear manganese complexes was attempted using the ligands TPDP and Me-TPDP. Synthesis of the TPDP ligated manganese complex, $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$ (**7**), under inert conditions, was successful. A reaction between **7** and O_2^- was observed resulting in the formation of a new species, **8**. **8** displayed electronic absorption, EPR, and X-ray absorption features remarkably similar to previously characterised $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complexes. Therefore, we postulated the formation of a new, unique $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complex. **8** was found to be unreactive towards the O-H bond containing substrate TEMPO-H and the one-electron reductant ferrocene.

Addition of 2 eq. of the acid *para*-toluenesulfonic acid (pTsOH) to **8** resulted in conversion to a new species **9**, monitored via UV-vis spectroscopy. EPR spectroscopy of **9** revealed a 21-line signal centred at $g \approx 2$, characteristic of a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -species distinct from **8**. XAS investigations showed an Mn K-edge energy shift of just 0.1 eV from **8** to **9**, consistent with both species having $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ oxidation states. Interestingly, there was a sharp decrease in the pre-edge area of **9** in comparison to **8**. This pre-edge behaviour has been observed previously in Mn^{III} -peroxides upon conversion to Mn^{III} -hydroperoxo species after addition of an acid. Therefore, we assigned **9** to be a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species, the first of its kind to be characterised via UV-vis, EPR and X-ray absorption spectroscopies. **9** was found to react slowly with the TEMPO-H substrate and post-reaction mixture EPR spectroscopy revealed the formation of the TEMPO $\cdot$ radical product. Demonstrating that **9** was capable of reacting with weak O-H bonds.

6.7 Experimental

Materials

All reactions with air-sensitive materials were performed in a glove box in a N₂ atmosphere or under inert conditions using inert Schlenk line techniques. All reagents and solvents were purchased from commercial sources. Anhydrous *N,N*-dimethylformamide (DMF) was purchased and used without further purification. Anhydrous tetrahydrofuran (THF), acetonitrile (MeCN), and acetone were dispensed through an MBraun MB-SPS-5 Solvent Purification System and deoxygenated by purging with argon.

Caution: KO₂ and perchlorate salts of metal complexes are potentially explosive and must be handled with care and in small quantities. No problems were encountered during this project.

Physical Methods

¹H NMR analysis was performed on a Bruker Avance III 400 MHz instrument. Electrospray ionisation mass spectra (ESI-MS) were obtained using micromass time of flight spectrometer (TOF), interfaced to a Waters 2690 HPLC, or by direct injection in the mass spectrometry instrument. Electronic absorption spectra were recorded in quartz cuvettes on a Hewlett Packard (Agilent) 8453 diode array spectrophotometer (190 – 1100 nm range) coupled to a liquid nitrogen cooled cryostat from Unisoku Scientific Instruments (Osaka, Japan). Electronic paramagnetic resonance (EPR) measurements were conducted on a Bruker Eleksys E-500 spectrometer with an Oxford ESR 910 liquid helium cryostat and an Oxford temperature controller. The 30 K EPR quantification of the signals were relative to a [Cu(NO₃)₂] spin standard in CH₃OH. The 77 K EPR quantifications of the signals were relative to a TEMPO• standard in 1:9 CH₃CN/THF.

X-ray absorption spectroscopy methods: The Mn-K-edge X-ray absorption data were collected on beamline 7-3 of the Stanford Synchrotron Radiation Lightsource (SLAC National Accelerator Lab, Menlo Park, CA, USA). Data were collected with the storage ring operating at 3.0 GeV and 500 mA, using a LN₂ cooled Si(220), $\phi = 90^\circ$ double-crystal monochromator, calibrated by using the first inflection point of a Mn foil (6539.0 eV). The monochromator was detuned by ~50% for higher harmonic rejection. A Canberra 32-element solid state germanium detector was used for fluorescence detection. All the measurements were performed at ambient pressure at ~17 K using an Oxford helium cryostat that was cooled by closed-cycle He gas loop. The parameters used for the scans were the following: 10 eV steps/1 second integration time in the pre-edge region, 0.3 eV steps /2 second integration time in the edge, and 0.05k steps in the EXAFS, with integration time increasing in a k^2 -weighted fashion from 2 to 4 seconds over the energy range ($k_{\text{max}} = 12.1k$). The total detector counts were typically 3-7k, well within the linear range of the detector electronics. Each sample was monitored for photoreduction. Delrin ® Mossbauer/XAS cups with sample windows of 4 mm x 10 mm. Elaboration of the XAS data, including averaging, background removal and normalization, was performed using Athena.⁴³ Edge energies were obtained by taking the first derivative of the rising edge, while pre-edge energies were identified using the second derivative. Peak fitting analysis employed the program Fityk,⁴⁴ using the protocol developed by Westre et al. for analyzing Fe K-edge pre-edge peaks.²⁸ Briefly, a 15-18 eV segment of the normalized $\mu(E)$ spectrum was fit around the pre-edge, with pre-edge peaks modeled using pseudoVoigt functions having a fixed 50:50 mixture of Gaussian:Lorentzian character, while the background absorption was modeled with a single pseudo-Voigt function where the proportion of Gaussian:Lorentzian character was allowed to float. Four slightly different energy ranges were fit separately, and the results averaged to generate the reported peak fits.

General Procedure for the reaction of 7 with cryptand

A 1:9 CH₃CN/THF solution (1.5 mM) of complex **7** was prepared. In a quartz cuvette 2 mL of a 1.5 mM solution of complex **7** was cooled to -90 °C. KO₂ (0.0056 g, 0.04 M) and cryptand (0.06 g, 0.08 M) were dissolved in DMF (2 mL). 75 µL of this solution was added to the solution containing **7**. The reaction progress was monitored using electronic absorption spectroscopy.

EPR Sample Preparation

Samples for EPR analysis were prepared by transferring ~ 1 mL of the desired solution from the quartz cuvette at -90 °C, via a pre-cooled pipette into a pre-cooled EPR tube and immediately freezing it in liquid nitrogen. The EPR spectra were recorded at 2 K (9.64 GHz, 0.2 mW microwave power).

*Synthesis of Mn^{II}₂(TPDP)(O₂CC₆H₅)₂(BPh₄) (**7**)*

The procedure is adapted from the synthesis of an analogous iron complex.⁴⁵ Under an inert atmosphere, benzoic acid (0.07 g, 0.54 mmol) and triethylamine (0.4 ml, 2.9 mmol) were added to a solution of TPDP ligand (0.24 g, 0.52 mmol) dissolved in degassed MeOH (23 ml). Via cannula transfer, the TPDP solution was added to solid Mn₂(OCCH₃)₂(H₂O)₄ (0.13 g, 0.57 mmol) and allowed to stir for 20 minutes. NaBPh₄ (0.36 g, 1.0 mmol) was dissolved in degassed MeOH (23 mL) and added via cannula to the TPDP solution yielding a yellow suspension after five minutes of stirring. The suspension was filtered and the solvent removed under vacuum from the filtrate to produce a brown powder. The powder was dissolved in the minimum acetonitrile and stored at -30 °C under inert atmosphere for one week to yield a resulting yellow/brown crystals. Yield (0.012g, 2%)

ATR-IR: ν[cm⁻¹] = 3057, 1601, 1562, 1479, 1385, 1293, 1154, 704

ESI-MS (m/z): Found m/z = 805.1790 ([M]⁺, C₄₁H₃₉Mn₂N₆O₅ requires 805.1743)

6.8 References

- (1) Eriksson, M.; Jordan, A.; Eklund, H. *Biochemistry* **1998**, *37* (38), 13359–13369.
- (2) Cotruvo, J. A.; Stubbe, J. *Annu. Rev. Biochem.* **2011**, *80* (1), 733–767.
- (3) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, *135* (10), 4027–4039.
- (4) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, *57* (4), 918–922.
- (5) Magherusan, A. M.; Kal, S.; Nelis, D. N.; Doyle, L. M.; Farquhar, E. R.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2019**, *58* (17), 5718–5722.
- (6) Pal, S. *Pyridine* **2018**.
- (7) Thies, S.; Bornholdt, C.; Köhler, F.; Sönnichsen, F. D.; Näther, C.; Tucek, F.; Herges, R. *Chem. - A Eur. J.* **2010**, *16* (33), 10074–10083.
- (8) Trehoux, A.; Roux, Y.; Guillot, R.; Mahy, J. P.; Avenier, F. *J. Mol. Catal. A Chem.* **2015**, *396*, 40–46.
- (9) Dong, Y.; Ménage, S.; Brennan, B. A.; Elgren, T. E.; Jang, H. G.; Pearce, L. L.; Que, L. *J. Am. Chem. Soc.* **1993**, *115* (5), 1851–1859.
- (10) Blanchard, S.; Blondin, G.; Rivière, E.; Nierlich, M.; Girerd, J. J. *Inorg. Chem.* **2003**, *42* (15), 4568–4578.
- (11) Teilum, K.; Olsen, J. G.; Kragelund, B. B. *Cell. Mol. Life Sci.* **2009**, *66* (14), 2231–2247.
- (12) Blanchard, S.; Blondin, G.; Rivière, E.; Nierlich, M.; Girerd, J.-J. *Inorg. Chem.* **2007**, *42* (15), 4568–4578.
- (13) Boal, A. K.; Cotruvo, J. A.; Stubbe, J.; Rosenzweig, A. C. *Biochemistry* **2012**, *51* (18), 3861–3871.
- (14) Boal, A. K.; Cotruvo, J. A.; Stubbe, J.; Rosenzweig, A. C. *Science* **2010**, *329* (5998), 1526–1530.
- (15) Teutloff, C.; Schäfer, K. O.; Sinnecker, S.; Barynin, V.; Bittl, R.; Wieghardt, K.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **2005**, *43* (SPEC. ISS.), 51–64.
- (16) Diril, H.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S.; Chang, H. R.; Nilges, M. J.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, *111* (14), 5102–5114.
- (17) Diril, H.; Chang, H. R.; Zhang, X.; Larsen, S. K.; Potenza, J. A.; Pierpont, C. G.; Schugar, H. J.; Isied, S. S.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1987**, *109* (20), 6207–6208.
- (18) Magherusan, A. M.; Zhou, A.; Farquhar, E. R.; García-Melchor, M.; Twamley, B.; Que, L.; McDonald, A. R. *Angew. Chemie - Int. Ed.* **2018**, *57* (4), 918–922.
- (19) Shook, R. L.; Gunderson, W. A.; Greaves, J.; Ziller, J. W.; Hendrich, M. P.; Borovik, A. S. *J. Am. Chem. Soc.* **2008**, *130* (28), 8888–8889.
- (20) Coggins, M. K.; Sun, X.; Kwak, Y.; Solomon, E. I.; Rybak-Akimova, E.; Kovacs, J. A. *J. Am. Chem. Soc.* **2013**, *135* (15), 5631–5640.
- (21) Kitajima, N.; Komatsiizaki, H.; Hikichi, S.; Osawa, M.; Moro-oka, Y. *J. Am. Chem. Soc.* **1994**, *116* (25), 11596–11597.
- (22) Liu, J. G.; Ohta, T.; Yamaguchi, S.; Ogura, T.; Sakamoto, S.; Maeda, Y.; Naruta, Y. *Angew. Chemie - Int. Ed.* **2009**, *48* (49), 9262–9267.
- (23) Liu, J. G.; Shimizu, Y.; Ohta, T.; Naruta, Y. *J. Am. Chem. Soc.* **2010**, *132* (11), 3672–3673.
- (24) Cho, J.; Sarangi, R.; Kang, H. Y.; Lee, J. Y.; Kubo, M.; Ogura, T.; Solomon, E. I.; Nam, W. *J. Am. Chem. Soc.* **2010**, *132* (47), 16977–16986.

- (25) Schreiber, R. E.; Cohen, H.; Leitus, G.; Wolf, S. G.; Zhou, A.; Que, L.; Neumann, R. *J. Am. Chem. Soc.* **2015**, *137* (27), 8738–8748.
- (26) Weng, T. C.; Hsieh, W. Y.; Uffelman, E. S.; Gordon-Wylie, S. W.; Collins, T. J.; Pecoraro, V. L.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **2004**, *126* (26), 8070–8071.
- (27) Rose, H. R.; Ghosh, M. K.; Maggiolo, A. O.; Pollock, C. J.; Blaesi, E. J.; Hajj, V.; Wei, Y.; Rajakovich, L. J.; Chang, W. C.; Han, Y.; Hajj, M.; Krebs, C.; Silakov, A.; Pandelia, M. E.; Bollinger, J. M.; Boal, A. K. *Biochemistry* **2018**, *57* (18), 2679–2693.
- (28) Westre, T. E.; Kennepohl, P.; DeWitt, J. G.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1997**, *119* (27), 6297–6314.
- (29) Warren, J. J.; Tronic, T. A.; Mayer, J. M. *Chem. Rev.* **2010**, *110* (12), 6961–7001.
- (30) Dubois, L.; Xiang, D. F.; Tan, X. S.; Pécaut, J.; Jones, P.; Baudron, S.; Le Pape, L.; Latour, J. M.; Baffert, C.; Chardon-Noblat, S.; Collomb, M. N.; Deronzier, A. *Inorg. Chem.* **2003**, *42* (3), 750–760.
- (31) Buchanan, R. M.; Oberhausen, K. J.; Richardson, J. F. *Inorg. Chem.* **1988**, *27* (6), 971–973.
- (32) Cox, N.; Ames, W.; Epel, B.; Kulik, L. V.; Rapatskiy, L.; Neese, F.; Messinger, J.; Wieghardt, K.; Lubitz, W. *Inorg. Chem.* **2011**, *50* (17), 8238–8251.
- (33) Sano, Y.; Weitz, A. C.; Ziller, J. W.; Hendrich, M. P.; Borovik, A. S. *Inorg. Chem.* **2013**, *52* (18), 10229–10231.
- (34) Goodson, P. A.; Hodgson, D. J.; Glerup, J.; Michelsen, K.; Pedersen, E. *Inorg. Chem.* **1990**, *29* (3), 503–508.
- (35) Frapart, Y. M.; Boussac, A.; Albach, R.; Anxolabéhère-Mallart, E.; Delroisse, M.; Verlhac, J. B.; Blondin, G.; Girerd, J. J.; Guilhem, J.; Cesario, M.; Rutherford, A. W.; Lexa, D. *J. Am. Chem. Soc.* **1996**, *118* (11), 2669–2678.
- (36) Sano, Y.; Weitz, A. C.; Ziller, J. W.; Hendrich, M. P.; Borovik, A. S. *Inorg. Chem.* **2013**, *52* (18), 10229–10231.
- (37) So, H.; Park, Y. J.; Cho, K. Bin; Lee, Y. M.; Seo, M. S.; Cho, J.; Sarangi, R.; Nam, W. *J. Am. Chem. Soc.* **2014**, *136* (35), 12229–12232.
- (38) Wijeratne, G. B.; Corzine, B.; Day, V. W.; Jackson, T. A. *Inorg. Chem.* **2014**, *53* (14), 7622–7634.
- (39) Borovik, A. S.; Que, L.; Papaefthymiou, V.; Taylor, L. F.; Anderson, O. P. *J. Am. Chem. Soc.* **1989**, *111* (16), 6183–6195.
- (40) Raycroft, M. A. R.; Maxwell, C. I.; Oldham, R. A. A.; Andrea, A. S.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2012**, *51* (19), 10325–10333.
- (41) Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, *39* (16), 3526–3536.
- (42) Suzuki, M.; Mikuriya, M.; Murata, S.; Uehara, A.; Oshio, H.; Kida, S.; Saito, K. *Bull. Chem. Soc. Jpn.* **1987**, *60* (12), 4305–4312.
- (43) Ravel, B.; Newville, M. *J. Synchrotron Radiat.* **2005**, *12* (4), 537–541.
- (44) Wojdyr, M. *J. Appl. Crystallogr.* **2010**, *43* (5), 1126–1128.
- (45) Dong, Y.; Ménage, S.; Brennan, B. A.; Elgren, T. E.; Jang, H. G.; Pearce, L. L.; Que, L. *J. Am. Chem. Soc.* **1993**, *115* (5), 1851–1859.

Chapter 7: Conclusions and Future Work

7.1 Conclusions

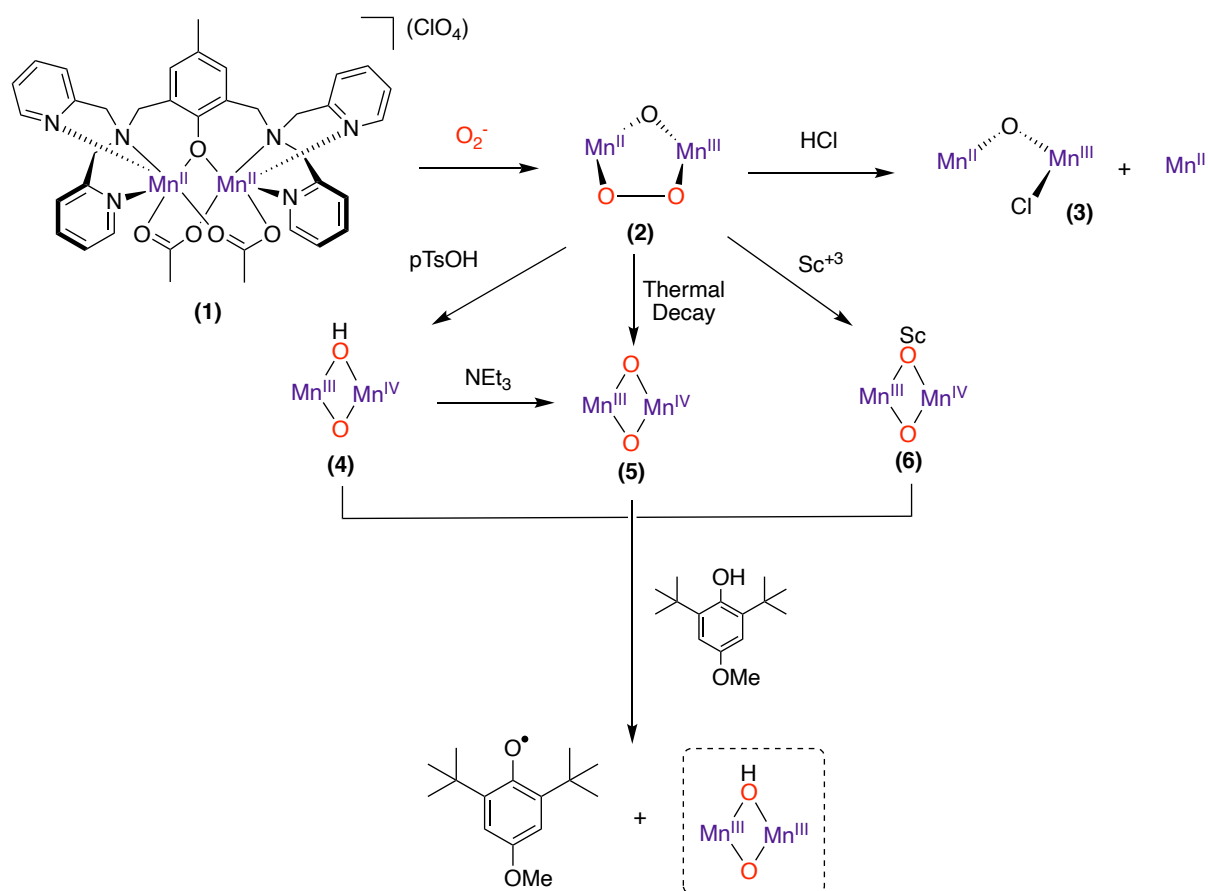


Figure 7.1: Summary of the species generated from $[\text{Mn}^{\text{II}}_2(\text{BPMP})(\text{OCCH}_3)_2](\text{ClO}_4)$ (**1**).

The aim of this thesis was to probe the mechanism of class Ib ribonucleotide reductases (RNRs) using model complexes of the enzyme active site. The biomimetic complexes were prepared and their reactivity with O_2^- and activation of subsequent species was explored. From the low-valent Mn^{II}_2 complex $(\text{Mn}^{\text{II}}_2(\text{OCCH}_3)(\text{BPMP}))(\text{ClO}_4)$, **1**, the metastable $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide species, **2**, was generated following reaction with O_2^- at -90°C (Figure 7.2). Species **2** was characterised via UV-vis, EPR, X-ray absorption spectroscopies and mass spectrometry. The yield of **2** was improved by 40 % when the chelating agent 18-crown-6, was substituted with 222-Cryptand. The reactivity of **2** with the aldehydes cyclohexanecarboxaldehyde (CCA) and phenylpropionaldehyde (PPA) was investigated

and **2** was found to be capable of aldehyde deformylation. Further kinetic analysis revealed that **2** displayed an exponential increase in k_{obs} with respect to an increase in aldehyde concentration. This indicated a non-linear mechanism during reaction of **2** with PPA and CCA. We postulated that the source of the exponential trend was initial reversible nucleophilic attack of the aldehyde by **2** followed by a Criegee rearrangement (Figure 7.2). The revelation that the $Mn^{II}Mn^{III}$ -peroxide species **2** could be activated using aldehyde substrates to perform nucleophilic reactivity revealed that $Mn^{II}Mn^{III}$ -peroxides were a promising candidate for the unknown intermediate species in the Ib RNR catalytic cycle. We decided to further explore the reactivity of **2** in an attempt to replicate the catalytic cycle of Ib RNRs and potentially learn more about the untrapped species.

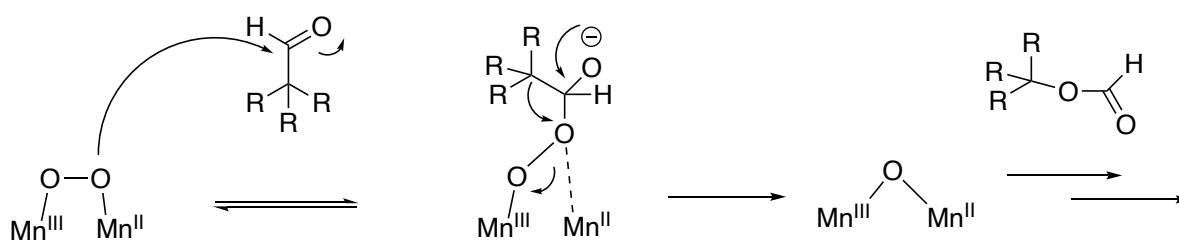


Figure 7.2: Nucleophilic attack of **2** on aldehyde substrates followed by an irreversible Criegee- rearrangement.

Following the synthesis and optimisation of the $Mn^{II}Mn^{III}$ -peroxide species **2** we were interested in probing its' reactivity with acids in order to understand the role, if any, of the proton in the catalytic cycle of Ib RNRs. Addition of the strong acid HCl to **2** resulted in the formation of a new species **3**. The electronic absorption features of **3** were very similar to previously characterised $Mn^{II}Mn^{III}$ complexes, however, they were shifted to lower energies. We postulated that the coordination of the Cl ion (derived from HCl) to the Mn centres was the source of the shift. Therefore, we tentatively postulated that the identity of **3** to be a $Mn^{II}Mn^{III}$ species with a coordinated Cl ion (Figure 7.3). EPR spectroscopy was

performed on frozen samples of **3** to understand the identity of the metal centres, however, the spectra were saturated by an intense six-line signal originating from free Mn^{II} ions. Reaction of **2** with the weak acid *para*-toluenesulfonic acid (pTsOH) resulted in the formation of a new species (**4**) with an intense feature at $\lambda_{\text{nm}} = 550$ nm in the UV-vis spectrum. New species **4** was further characterised using EPR and X-ray absorption spectroscopies and mass spectrometry and determined to be a new bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. Base reactivity studies with **4** indicated that a proton may reside on the bis(μ -oxo)core of the complex. Complex **4** reacted with the O-H bond containing substrate 4-methoxy-2,6-di-*tert*-butylphenol (4-OCH₃-2,6-DTBP). The EPR spectroscopy investigation into the post-reaction mixture revealed an intense isotropic signal centred around $g = 2$, which indicated that **4** had completed one-electron oxidation of 4-OCH₃-2,6-DTBP to generate a phenoxyl radical species (Figure 7.3). Further kinetic investigations of **4** with various O-H bond containing substrates determined that **4** utilised a HAT mechanism during oxidation.

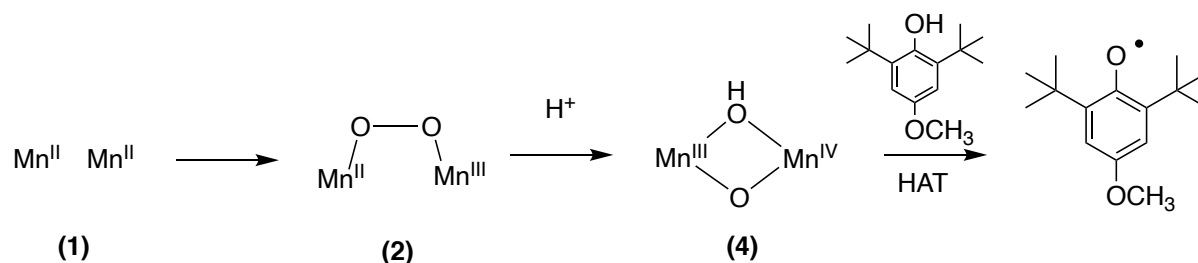


Figure 7.3: Formation of complex **4** and its reaction with 4-OCH₃-2,6-DTBP.

The work in Chapter 3 demonstrated that addition of a strong vs weak acid to **2** resulted in the formation of two different species. We postulate that addition of HCl resulted in the breaking of the Mn-O bond and the generation a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ species with formation of H_2O_2 . On the other hand, addition of the weak acid species pTsOH resulted in O-O bond cleavage and formation of a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. This demonstrated that it was possible for a proton

to initiate the conversion from a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide to $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$, however, the strength of the acid was important in determining product formed. This was of great relevance to Ib RNRs as it illustrated that the origin of the postulated proton in the catalytic cycle by Stubbe *et al.* is important, otherwise the demetallation of the metallocofactor may occur.¹

$\text{Bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes have been observed as thermal decay products of Mn^{III} -peroxides, therefore, we were interested in investigating the effect of warming $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complex **2**. Warming of **2** from $-90\text{ }^{\circ}\text{C}$ to $+20\text{ }^{\circ}\text{C}$ resulted in the formation of a new broad, featureless electronic absorption spectrum that tailed into near-IR region associated with the formation of a new species (**5**). Further EPR, XAS, and mass spectrometry investigations confirmed complex **5** possessed a $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ structure. Base reactivity studies revealed that **5** contained a “naked” $\text{bis}(\mu\text{-oxo})$ core. This was in contrast to species the $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species **4** where base studies indicated that the core of **4** contained a proton.

Reactivity studies of **5** demonstrated that this new species was also capable of performing one-electron oxidation of O-H bond containing phenols. The EPR spectrum of the post reaction mixture of **5** and 4-OCH₃-2,6-DTBP also revealed an intense isotropic signal centred at $g = 2$, characteristic of the formation of the corresponding phenoxyl radical species, similar to that observed following the reaction of **4** and the same substrate. Kinetic investigation of the reaction of **5** and 4-OCH₃-2,6-DTBP revealed that **5** displayed saturation kinetics. The saturation behaviour was modelled as Michaelis-Menten type kinetics and indicated that complex **5** undergoes a two-step mechanism during the oxidation of phenols. We postulated that because complex **5** did not contain a proton on the $\text{bis}(\mu\text{-oxo})$ core, it was not electrophilic enough to directly attack the O-H bond of the phenol. Instead, it requires an initial activation step whereby the proton of the phenol associated

with the bis(μ -oxo) core of **5**, increasing the electrophilicity of the complex and activating it towards O-H bond attack (Figure 7.4). This was consistent with the observation of a linear mechanism in complex **4**, which we postulated contained a proton on the bis(μ -oxo) core. The presence of a proton on **4**, increased the electrophilicity of the core and negated the requirement for the association step required for activation (Figure 7.4). Further kinetic analysis of the reaction of **5** with O-H bond containing substrates lead to the discovery that **5** utilised a HAT mechanism during the oxidation.

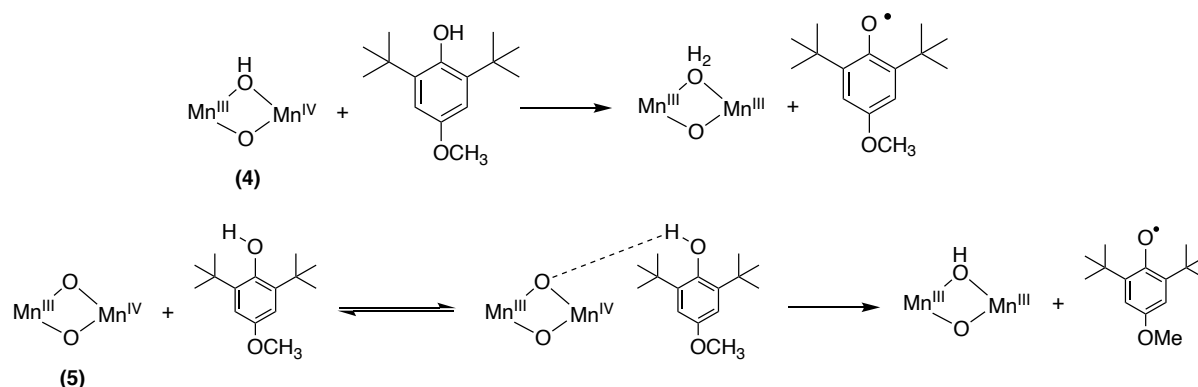


Figure 7.4: The different mechanisms used by complexes **4** and **5** during the reaction with O-H bond containing substrates.

Our discovery that Mn^{III}Mn^{IV} species **4** and **5** employed a HAT mechanism during the oxidation of O-H bonds had interesting implications for the catalytic cycle of Ib RNRs. The mechanism of oxidation employed by the Mn^{III}Mn^{IV} species of the tyrosine residue in the enzyme has never been studied. Therefore, we postulate that, in analogy with our biomimetic species, **4** and **5**, that the Mn^{III}Mn^{IV} of Ib RNRs utilised a HAT mechanism during O-H bond oxidation. A HAT mechanism was previously suggested for the enzyme as it would circumvent the step-wise formation of high-energy intermediates that could damage the protein structure.² Until now though, no experimental evidence has been provided for a HAT mechanism, therefore, our biological mimics **4** and **5** provided much needed insight into the oxidation process.

Moreover, the revelation that the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes **4** and **5** undergo a linear versus Michaelis-Menten reaction depending on the protonation state of the bis(μ -oxo) core was also pertinent to the catalytic cycle of Ib RNRs. The $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species responsible for tyrosine oxidation was postulated to be a (μ -hydroxo)(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species by Stubbe *et al.*¹ However, no experimental support for this structure exists, only a 16-line EPR signal confirming the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ oxidation states. Therefore, once the complete structure of the active species is identified, our work can shed light on if the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species reacts via a linear one-step reaction or a two-step Michaelis-Menten type reaction. Alternatively, if the kinetics of oxidation of the $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species in the enzyme was measured, the presence of linear versus Michaelis-Menten kinetics would indicate the protonation state of the core.

Due to our successes of activation of the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide using the proton source, pTsOH, we were interested in expanding to Lewis acid sources, such as scandium. Addition of 1 eq. of $\text{Sc}(\text{OTf})_3$ (= scandium trifluoromethanesulfonate) resulted in an immediate change in the electronic features of **2**, indicative of the formation of new species (**6**). **6** displayed two new electronic absorption features at $\lambda_{\text{max}} = 410$ and 550 nm. Further analysis of **6** via EPR, XAS and mass spectrometry revealed **6** to be a new bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species. **6** was also found to be capable of the one-electron oxidation of 4-OCH₃-2,6-DTBP via EPR spectroscopy. The kinetics of the reaction of **6** and 4-OCH₃-TEMPO-H (= 4-methoxy-1-hydroxy-2,2,6,6-tetramethyl-piperidine) were investigated under *pseudo* first-order conditions. **6** displayed a linear mechanism during the reaction with the O-H bond containing substrate. Comparison of the k_2 value for the reaction of **6** and **4** with the same substrate (4-OCH₃-TEMPO-H) at the same temperature revealed that **6** reacted four-times faster than **4**. We postulated that the increase in reactivity of complex **6** was due to the presence of a Sc^{3+} ion on the bis(μ -oxo) core, which is a stronger Lewis acid compared to

H⁺. The presence of Sc³⁺ on **6**, resulted in a more electrophilic core and greater reactivity compared to complex **4** (Figure 7.5). This also supported our postulate that the source of complex **5**'s saturation kinetics was its "naked" bis(μ -oxo) core. Moreover, complex **6** was the third Mn^{III}Mn^{IV} species generated via activation of the **2** and verified that Mn^{II}Mn^{III}-peroxide species are a prime candidate for the unknown intermediate species in the catalytic cycle of Ib RNRs.

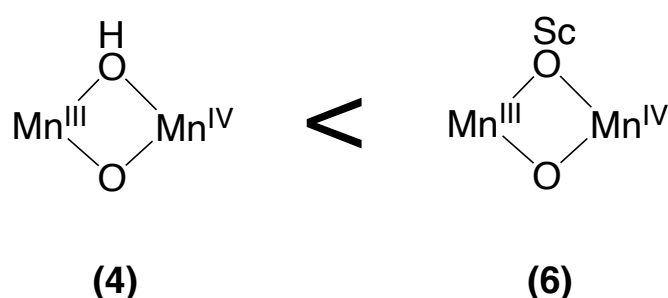
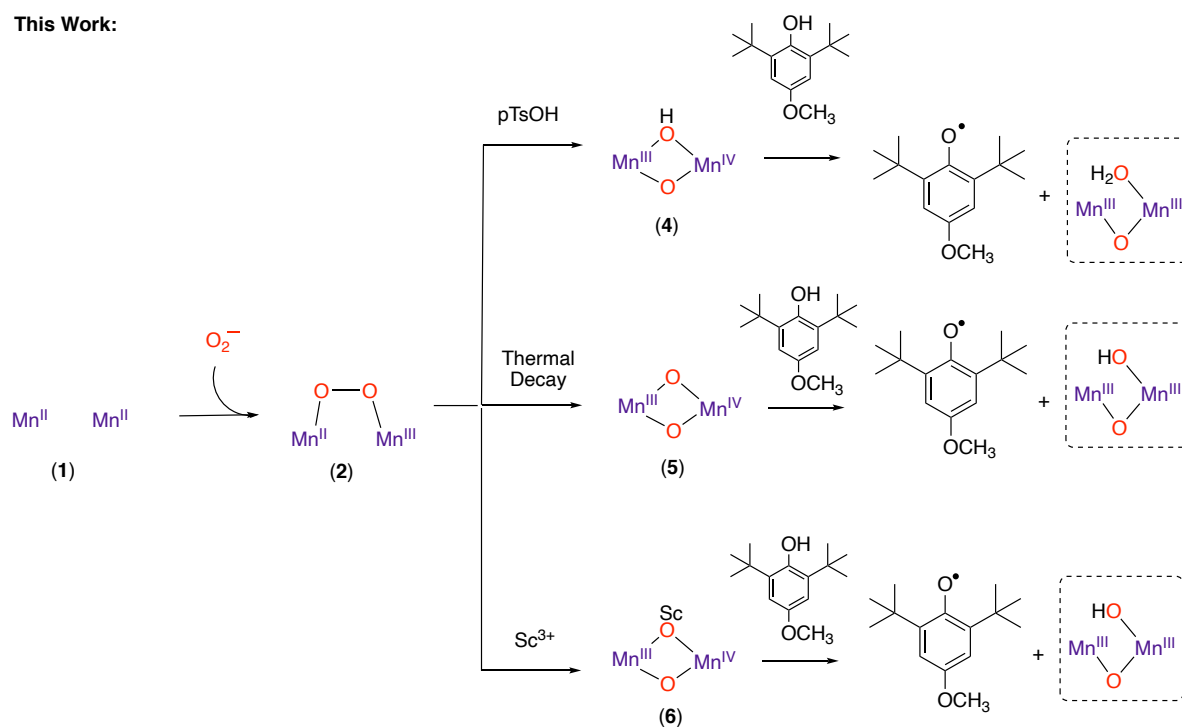


Figure 7.5: Complex **6** reacts faster with O-H bonds compared to complex **4**.

From the low-valent Mn^{II}₂ complex **1**, a Mn^{II}Mn^{III}-peroxide species (**2**) was generated upon addition of O₂^{•−}. Complex **2** displayed rich chemistry and could be activated via many different methods to afford a variety of species. Addition of strong acid resulted in the formation of a mixed valent Mn^{II}Mn^{III} species (**3**). **2** could also be converted to a bis(μ -oxo)Mn^{III}Mn^{IV} complex via addition of a proton source (**4**), thermal decay (**5**), or addition of a Lewis acid (**6**). All three complexes, displayed the ability to oxidise phenols to the corresponding radical species. This was similar to the reactivity observed in the catalytic cycle of Ib RNRs, whereby a Mn^{II}₂ site reacts with O₂^{•−} to generate an unknown intermediate that rapidly converted to a bis(μ -oxo)Mn^{III}Mn^{IV} species, responsible for oxidation of tyrosine to a tyrosyl radical species. Therefore, we have demonstrated the first case of the complete mimicry of the catalytic cycle of Ib RNRs (Figure 7.6).

From our biomimetic complexes a number of insights can be made into the function of Ib RNRs. For example, from our studies of the kinetics of the oxidation of O-H bonds by $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ we suggest that tyrosine oxidation occurs via a HAT pathway. Also, our discovery that $\text{bis}(\mu\text{-oxo})\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes reacted via a one-step or two-step mechanism depending whether or not the $\text{bis}(\mu\text{-oxo})$ core was protonated shed light on the role of the postulated proton in Ib RNR to activate the core towards O-H bond oxidation. However, one of the most important discoveries made in this work was that $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxides are a promising candidates for the unknown intermediate preceding $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ formation. In the enzyme, species has never been trapped and no spectroscopic evidence of its' structure exists. Nonetheless, in this work we have consistently shown that from a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide a number of $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species capable of the reactivity of Ib RNRs can be generated. Moreover, this $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide was formed under similar conditions as that of the enzyme. Therefore, this work has played a vital role in understanding the unknown species and completing the catalytic cycle of Ib RNRs.

This Work:



Proposed RNR Mechanism:

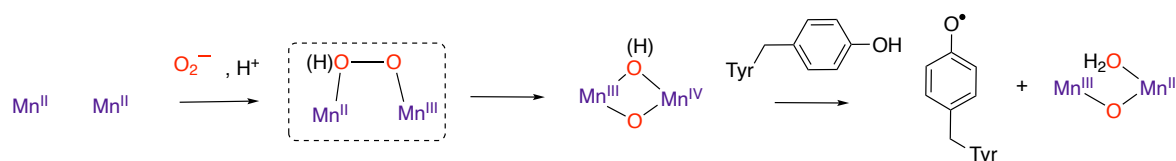


Figure 7.6: Upper: scheme of the reactivity of complexes **4**, **5**, and **6**. Lower: Proposed scheme for the catalytic cycle of Ib RNRs.¹ The dashed lines indicate the unknown intermediate and the involvement of a proton is postulated hence it is included in brackets.

We were also interested in probing the effects of tuning the ligand environment of the Mn centres. We set out to synthesise a new Mn^{II}_2 complex ($[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$ = **7**) and investigate its reactivity with superoxide. Complex **7** was synthesised and characterised via XRD, IR, NMR, XANES, and mass spectrometry methods and was found to contain two Mn^{II}_2 centres ligated by the TPDP ligand and two benzoic acid moieties (Figure 7.7).

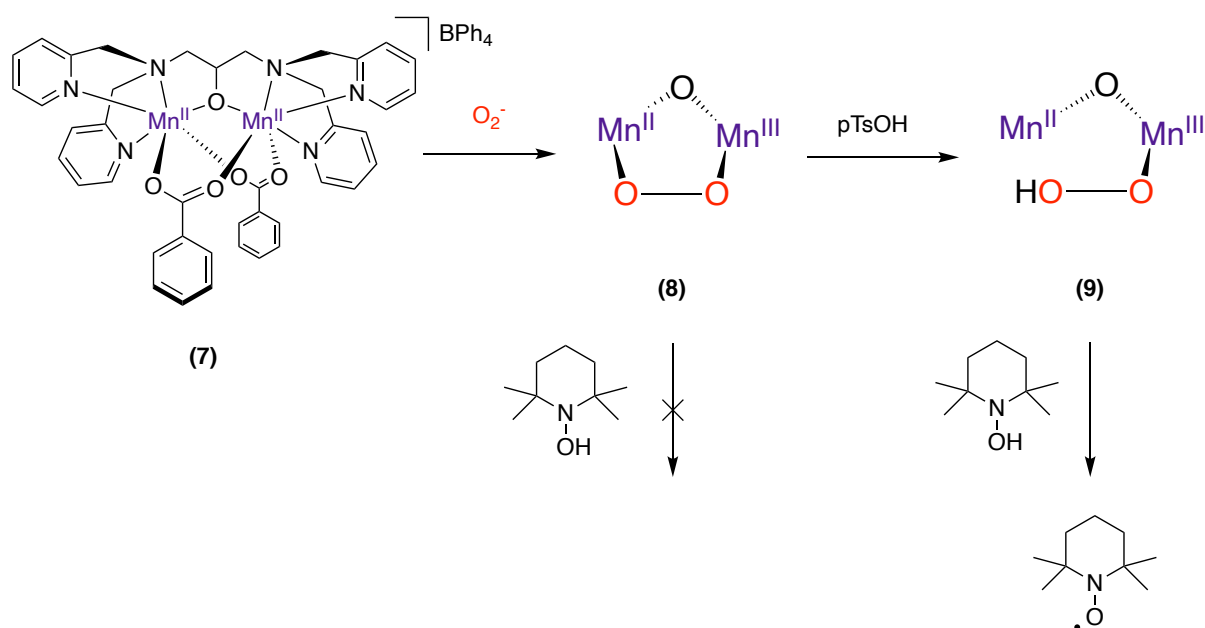


Figure 7.7: Summary of the species generated from [Mn^{II}₂(TPDP)(O₂CC₆H₅)₂](BPh₄) (**7**).

Interestingly, upon addition of 1 eq. of O₂⁻ to **7** at -90 °C, two new features were formed in the UV-vis spectrum at $\lambda_{\text{max}} = 450$ and 590 nm, associated with the formation of a new species, **8**. New species **8** was analysed via EPR, XAS, Raman, and mass spectrometry techniques and assigned as a new Mn^{II}Mn^{III}-peroxide species (Figure 7.6). This was, to the best of our knowledge, only the third Mn^{II}Mn^{III}-peroxide species to be characterised in the literature. Interestingly, all of the known Mn^{II}Mn^{III}-peroxides were generated upon reaction of Mn^{II}₂ with O₂⁻, indicating that Mn^{II}Mn^{III}-peroxides appeared to be thermodynamically favoured and provided further weight for this to be the unknown species in the catalytic cycle of Ib RNRs.

8 was found to be unreactive with the weak O-H bond containing substrate TEMPO-H (= 2,2,6,6-tetramethylpiperidin-1-ol, BDE = 70.6 kcal mol⁻¹) and the reducing agent ferrocene, demonstrating **8** to be a poor electrophile.³ Addition of 2 eq. of pTsOH to **8** resulted in the formation of new species **9** as evidenced by the change in the UV-vis features to a long, broad featureless spectrum. Complex **9** was characterised using EPR and XANES to be a

new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species (Figure 7.6). Interestingly, **9** was found to be capable of the oxidation of the O-H bond of TEMPO-H via EPR spectroscopy. By comparison to its precursor **8** displayed no reactivity with the same substrate. Despite this, the reaction of **9** and TEMPO-H was sluggish and afforded only modest yields of the TEMPO $\cdot$ radical. This was quite significant for the catalytic cycle of Ib RNRs as the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes **4**, **5**, and **6** were all extremely capable of oxidising the O-H bonds of the TEMPO-H substrates. However, the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo complex **9** struggled with this relatively weak substrate. This demonstrated why Ib RNRs employed a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species for the oxidation of the O-H bond of the tyrosine over a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species as they are much more capable oxidants.

In conclusion, the model complexes of the active site of Ib RNRs prepared in this thesis have provided invaluable insight into the structure and function of the intermediates in the catalytic cycle that cannot be trapped due to their fleeting lifetimes.

7.2 Future Work

From the results of this project, many routes exist to further explore the catalytic cycle of class I RNRs:

1. Briefly discussed during this project was the base chemistry that related the complexes **4** and **5** and allowed us to understand the protonation state of the bis(μ -oxo) cores of these complexes. Further work could expand on this and investigate if it is possible to convert between all three bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ species **4**, **5**, and **6**. Moreover, detailed acid-base studies of complex **4** could allow the pK_a of the O-H bond on the (μ -hydroxo)(μ -oxo)core to be calculated. This may allow us to further understand the protonation state of the bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex responsible for oxidation of the tyrosine residue in class Ib RNRs.

2. Density functional theory (DFT) analysis of the new complexes synthesised in this thesis will be essential for completely understanding of these new species. For example, DFT analysis of the of bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complexes **4**, **5**, and **6** would provide great insight into the mechanism of formation of these species from the precursor $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide complex **2**. Moreover, DFT could provide further weight to our postulate that a the “naked” bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ of complex **5** is the source of the saturation kinetics displayed by the complex and that the presence of H^+ and Sc^{3+} on **4** and **6** respectively allows these complexes to directly act the substrate without the requirement for oxidation. DFT could also allow us to understand why in the presence of the proton source pTsOH the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **2** converted to a bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ complex, whereas the related species **7** reacted with pTsOH to form a $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species. Understanding the factors which control bis(μ -oxo) $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ vs $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo formation could allow us to better understand the active site and catalytic cycle of Ib RNRs and design better biomimetic complexes.
3. We tentatively assign the formation of a new $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo species (**9**) in Chapter 6. Further work must be done to in order to completely characterise this new species. Raman spectroscopy of the $\nu_{\text{O-OH}}$ would provide valuable evidence for the formation of the first $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -hydroperoxo complex. Initial studies were performed on the $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ -peroxide **8**, however, the solvent systems masked the areas a $\nu_{\text{O-O}}$ stretch would be expected. Therefore, further Raman experiments should be performed in a variety of solvent systems in order to observe the $\nu_{\text{O-O}}$ and $\nu_{\text{O-OH}}$ stretches of **8** and **9** respectively. Preliminary studies of the reactivity of **9** were performed on TEMPO-H which contained a relatively weak O-H bond. However, an in-depth study of the reaction mechanism of would be of great interest

to compare to the similar analysed bis(μ -oxo) complexes. That is, to see if **9** reacted via the same HAT mechanism as complexes **4** and **5** or via an alternative pathway.

4. Great strides have been made in the past few years on understanding the mechanism of Ib RNRs using biomimetic complexes. This has emphasised the role that reactivity studies on model complexes play in understanding enzyme function. From the lessons learned in this project on Ib RNRs, it would be worth expanding to Id RNRs, an enzyme where even less is known about its catalytic cycle. Class Id RNRs also utilise a Mn^{II}_2 active site and react with O_2^- to form a high valent $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ active oxidant.⁴ However, very little is known about the intermediate species or the structure of the active oxidant. Due to the similarities between Ib and Id RNRs, the work in this thesis could act as a base to understand this recently discovered enzyme. Furthermore, even expanding to the Ic RNRs, the subclass that utilises a mixed metal (Fe and Mn) in the active site, would be worth pursuing.⁵ Understanding why this class of enzyme employed both Fe and Mn instead of just one type of metal and what benefits this afforded the enzyme would be of great interest.

7.3 References

- (1) Cotruvo, J. A.; Stich, T. A.; Britt, R. D.; Stubbe, J. *J. Am. Chem. Soc.* **2013**, *135* (10), 4027–4039.
- (2) Minnihan, E. C.; Nocera, D. G.; Stubbe, J. A. *Acc. Chem. Res.* **2013**, *46* (11), 2524–2535.
- (3) Warren, J. J.; Tronic, T. A.; Mayer, J. M. *Chem. Rev.* **2010**, *110* (12), 6961–7001.
- (4) Rose, H. R.; Ghosh, M. K.; Maggiolo, A. O.; Pollock, C. J.; Blaes, E. J.; Hajj, V.; Wei, Y.; Rajakovich, L. J.; Chang, W. C.; Han, Y.; Hajj, M.; Krebs, C.; Silakov, A.; Pandelia, M. E.; Bollinger, J. M.; Boal, A. K. *Biochemistry* **2018**, *57* (18), 2679–2693.
- (5) Jiang, W.; Yun, D.; Saleh, L.; Barr, E. W.; Xing, G.; Hoffart, L. M.; Maslak, M. A.; Krebs, C.; Bollinger, J. M. *Science* **2007**, *316* (5828), 1188–1191.

Appendix

Chapter 2 Appendix

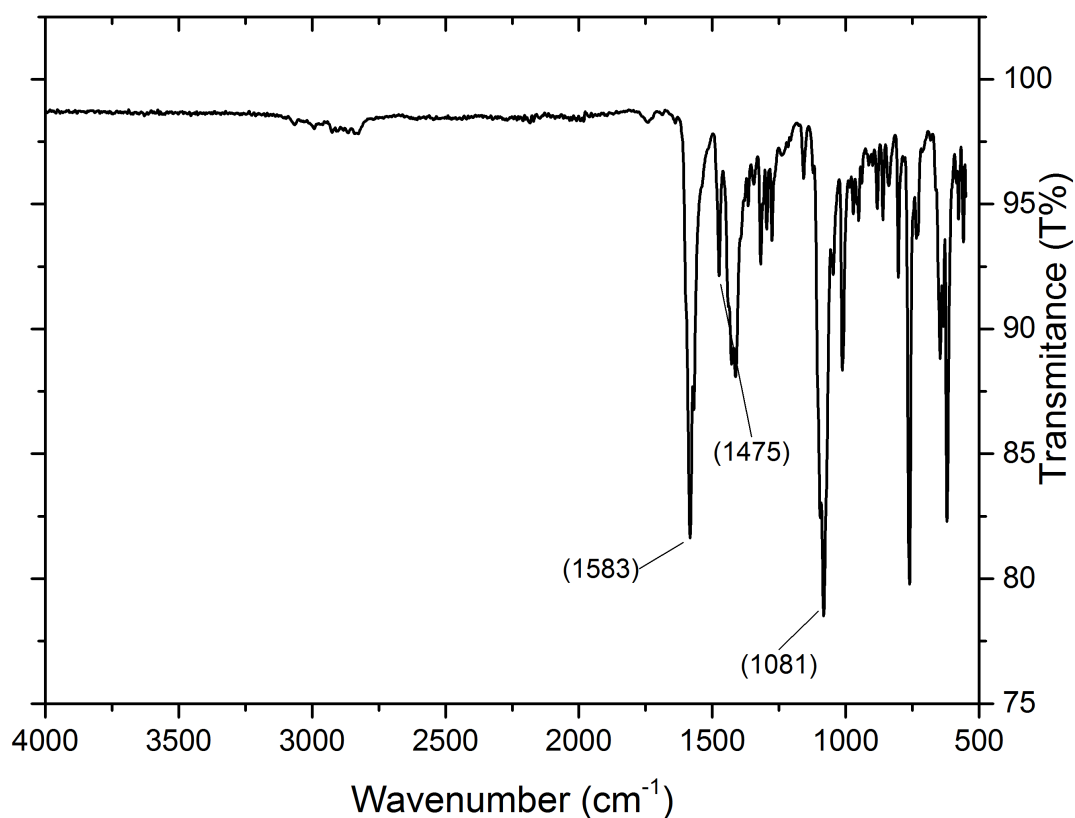


Figure A2.1: IR spectra of **1**. Highlighted bands correspond to the symmetric and asymmetric stretching of the bridging acetates (1583 cm^{-1} and 1475 cm^{-1}) and the presence of a perchlorate counter ion (1081 cm^{-1}).

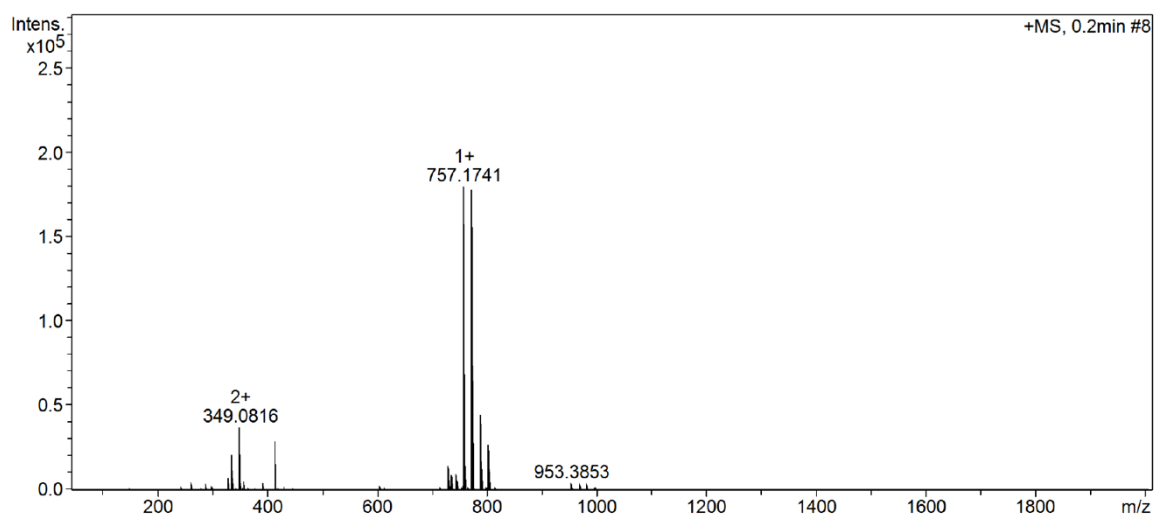


Figure A2.2: ESI-mass spectrum of the complex $[\text{Mn}_2^{\text{II}}(\text{BPMP})(\text{OCCH}_3)](\text{ClO}_4)$ (**1**). Peak m/z = 757.1741 associated with the cation $[\text{Mn}_2^{\text{II}}(\text{BPMP})(\text{OCCH}_3)]^+$.

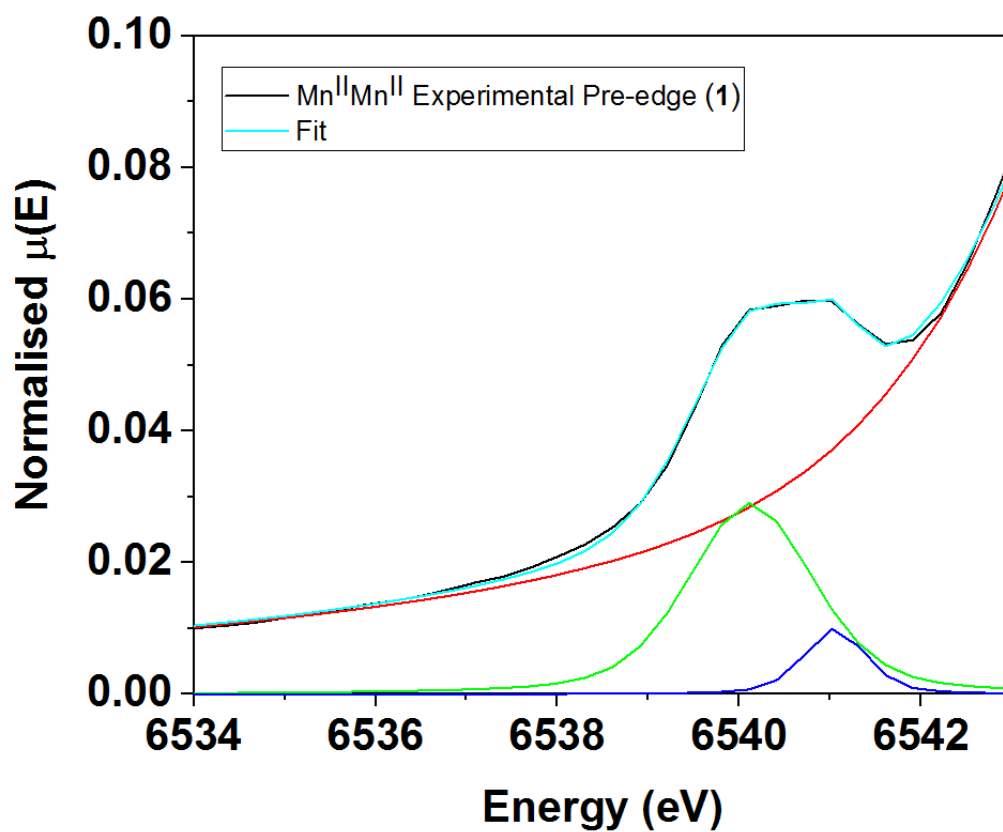


Figure A2.3: Rising edge and peak fitting of the pre-edge area of 1 with two 50:50 pseudo-Voigt function.

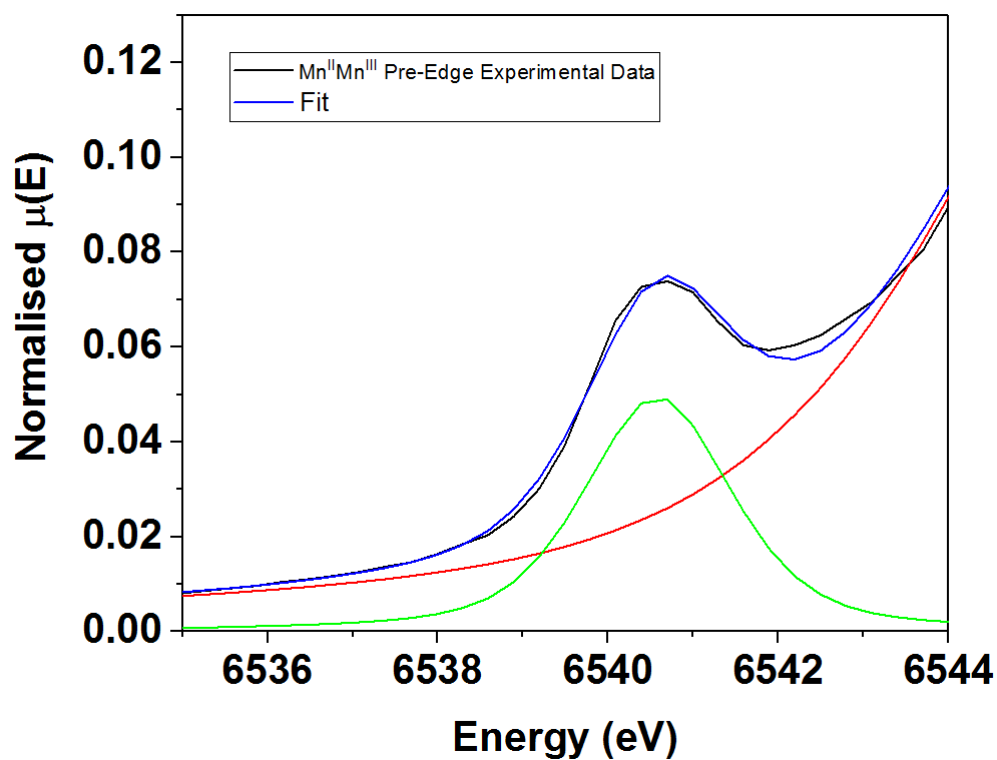


Figure A2.4: Rising edge and peak fitting of the pre-edge area of 2 with a 50:50 pseudo Voigt function.

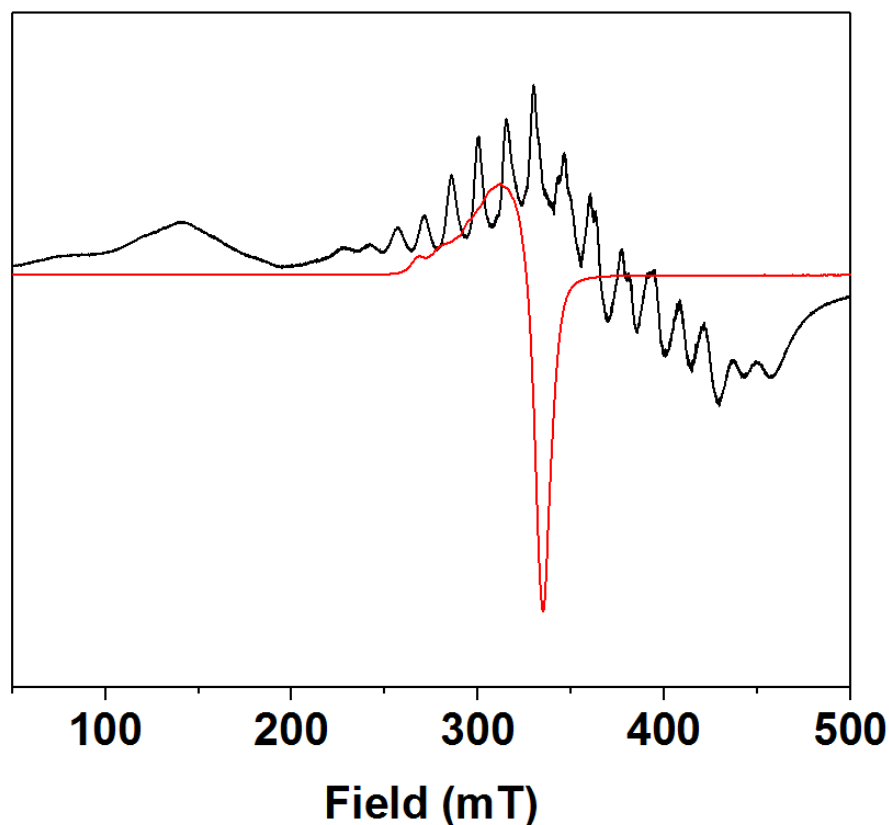


Figure A2.5: Perpendicular mode EPR spectra of **2** in 1:9 CH₃CN/THF (obtained from the reaction of 1.5 mM **1** with KO₂ in 1:9 CH₃CN/THF-black line) and Cu(NO₃)₂ standard (0.25 mM) in CH₃OH (red line) (2 K, 9.64 GHz, 0.2 mW microwave power).

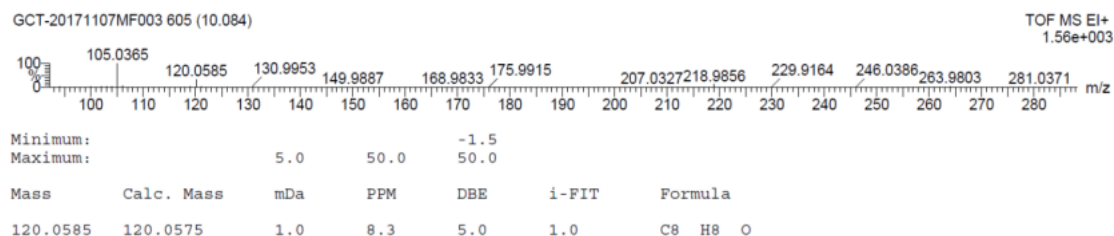


Figure A2.6: Mass spectrometry evidence of the formation of acetophenone ($m/z = 120.585$) from the post-reaction mixture of **2** and PPA

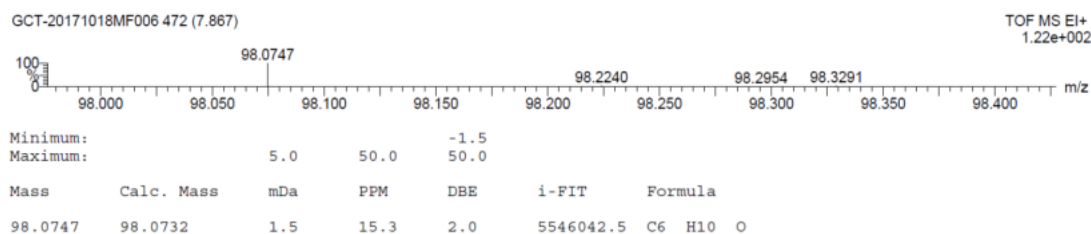


Figure A2.7: Mass spectrometry evidence of the formation of cyclohexanone ($m/z = 98.0723$) in the post-reaction mixture of **2** and CCA.

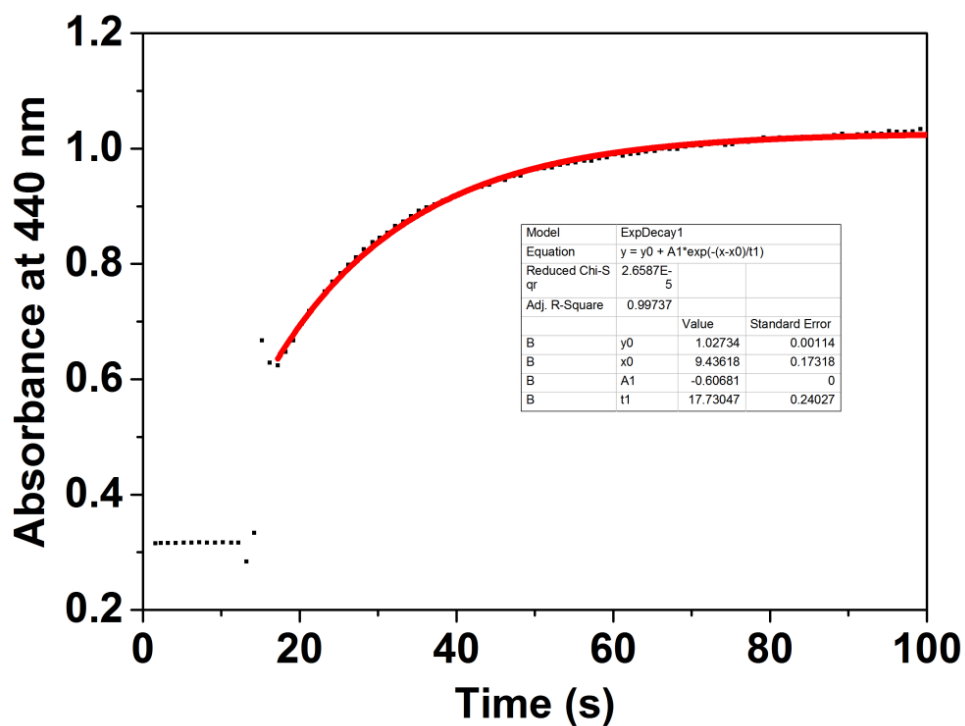


Figure A2.8: Plot in the change of absorbance at 440 nm during the reaction of **2** and CCA (black trace). Pseudo first-order fit of the resulting curve (red trace).

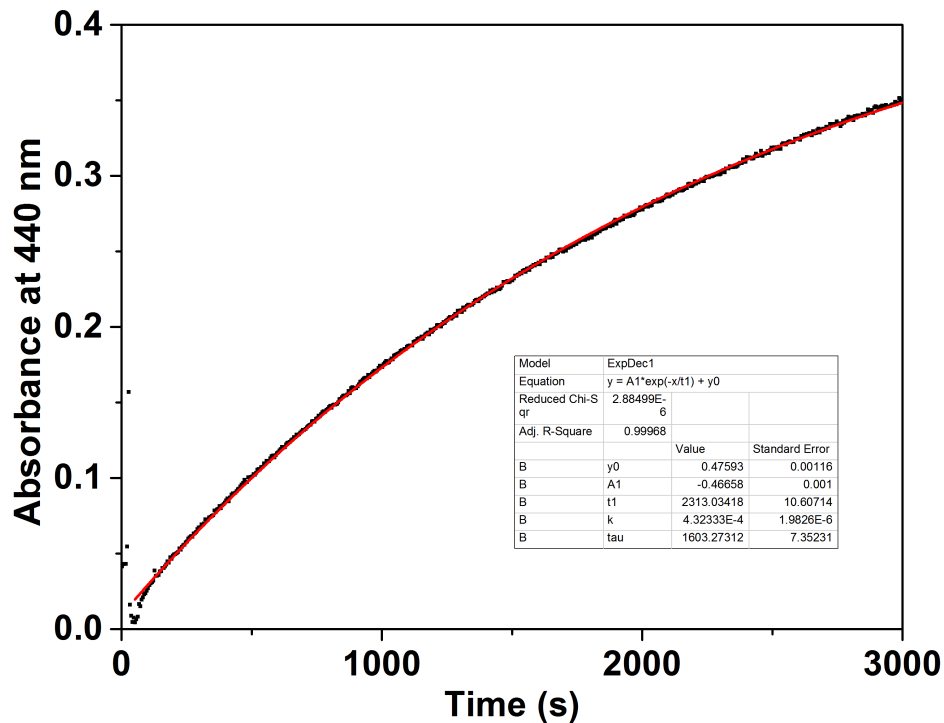


Figure A2.9: Plot in the change of absorbance at 440 nm during the reaction of **2** and PPA (black trace). Pseudo first-order fit of the resulting reaction (red trace).

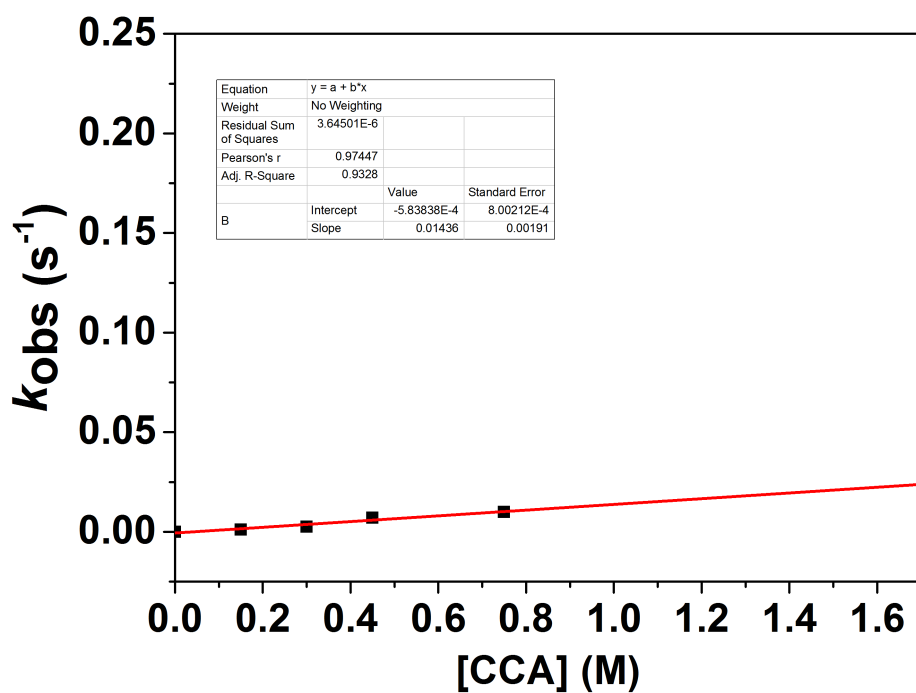


Figure A2.10: Plot of k_{obs} versus low concentration CCA region determined for the reaction between 2 and CCA.

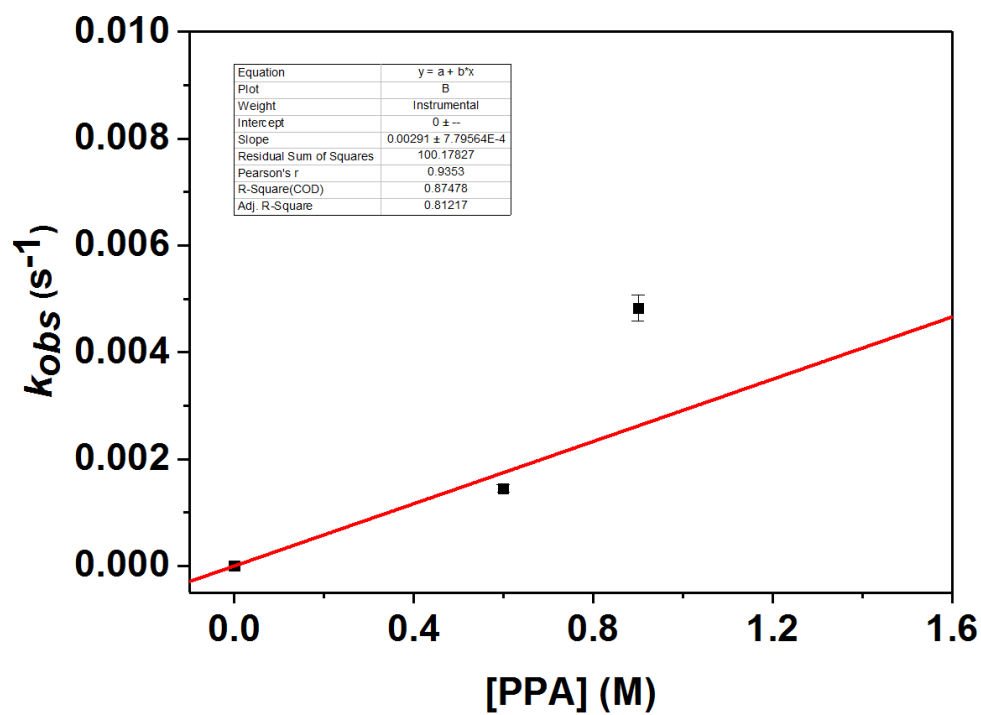


Figure A2.11: Plot of k_{obs} versus low concentration PPA region determined for the reaction between 2 and PPA.

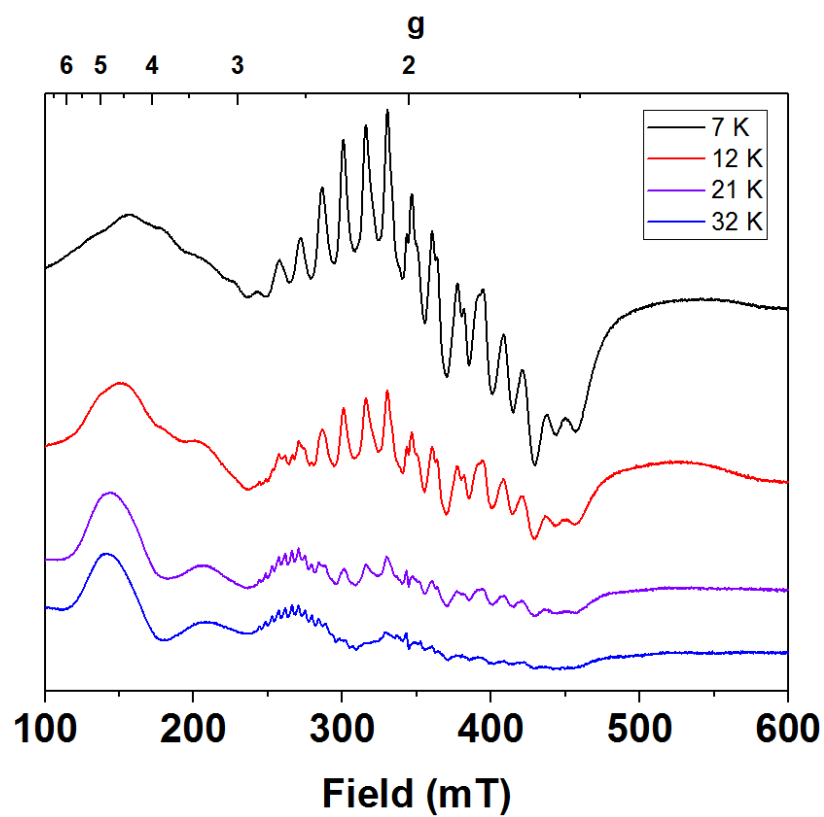


Figure A2.12: Perpendicular X-Band EPR spectrum of **2** (generated from 1.5 mM Mn_2BPMP + 1 eq. KO_2) measured at 7 K (black), 12 K (red), 21 K (purple), 32 K (blue) (9.65 GHz, 0.2 mW microwave power).

Chapter 3 Appendix

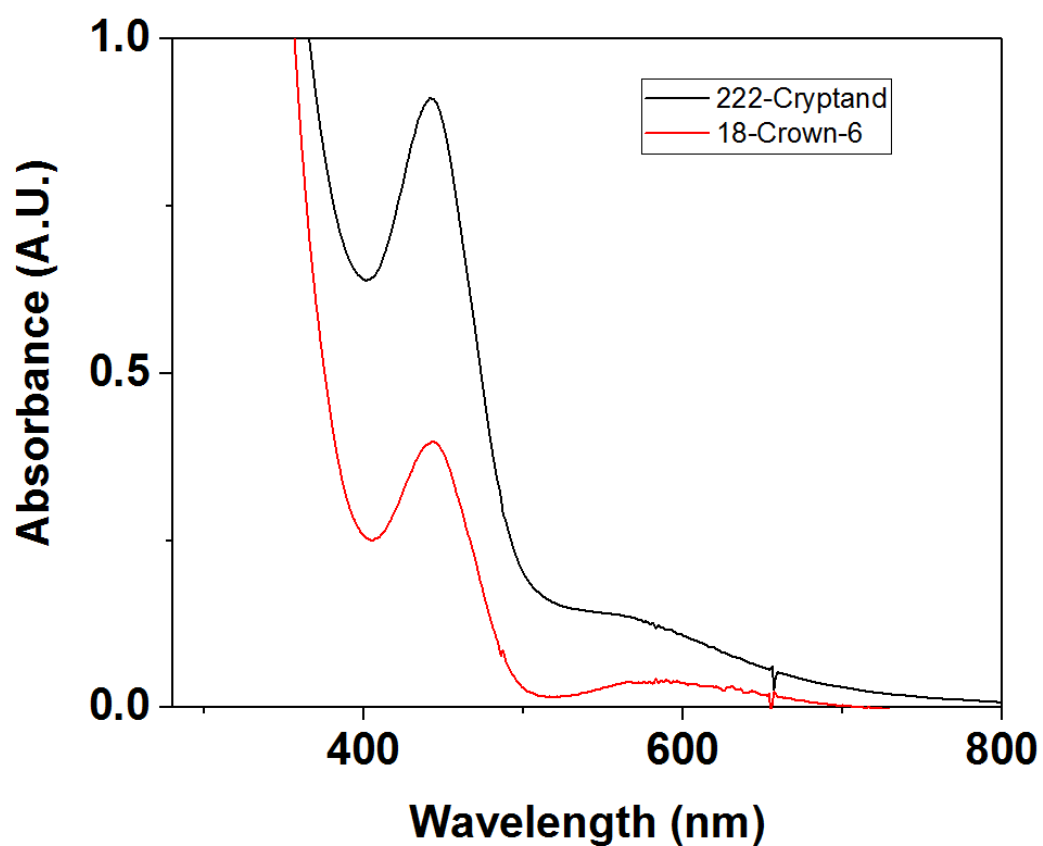


Figure A3.1: Electronic absorption spectra of **2** generated using 222-Cryptand (black trace) and 18-crown-6 (red trace).

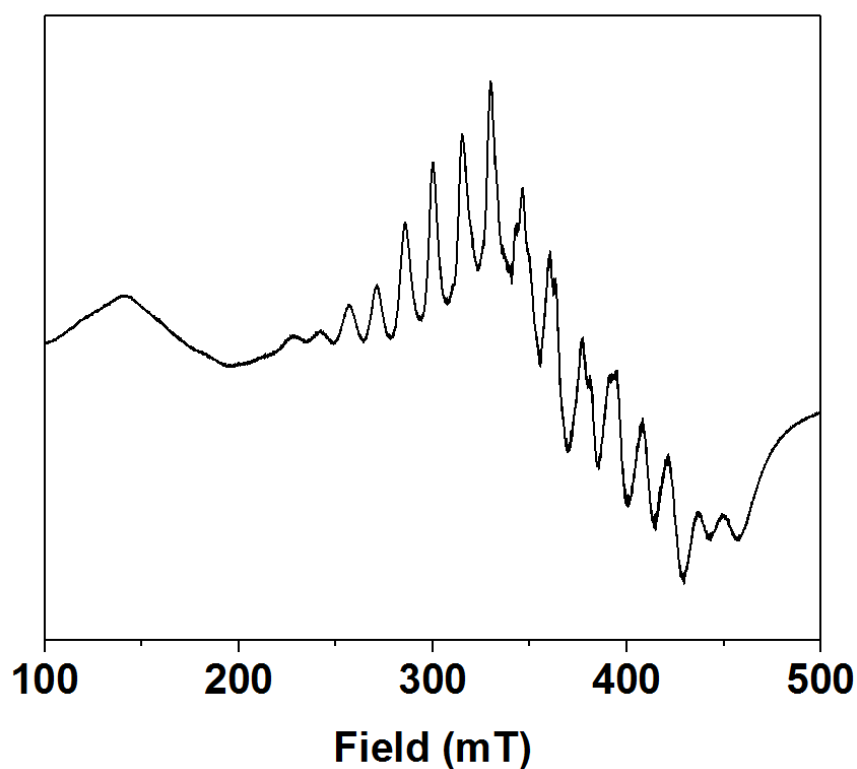


Figure A3.2: Perpendicular mode X-band EPR spectrum of **2** at 2K (black trace) obtained from the reaction of **1** (1.5 mM) and KO_2 solubilised with 222-Cryptand in 1:9 $\text{CH}_3\text{CN}:\text{THF}$ (9.64 GHz, 0.2 mW microwave power)

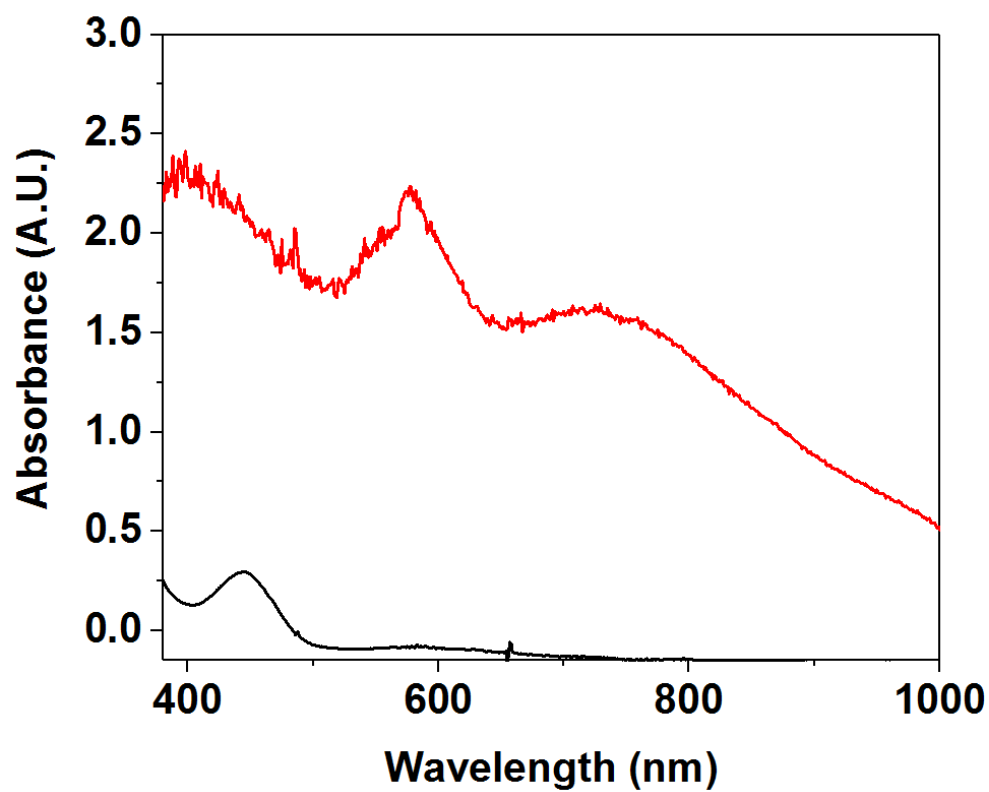


Figure A3.3: Electronic absorption spectra of the reaction of 2 (black trace) + 4 eq. HCl (red) in 1:9 MeCN/THF at -90 °C.

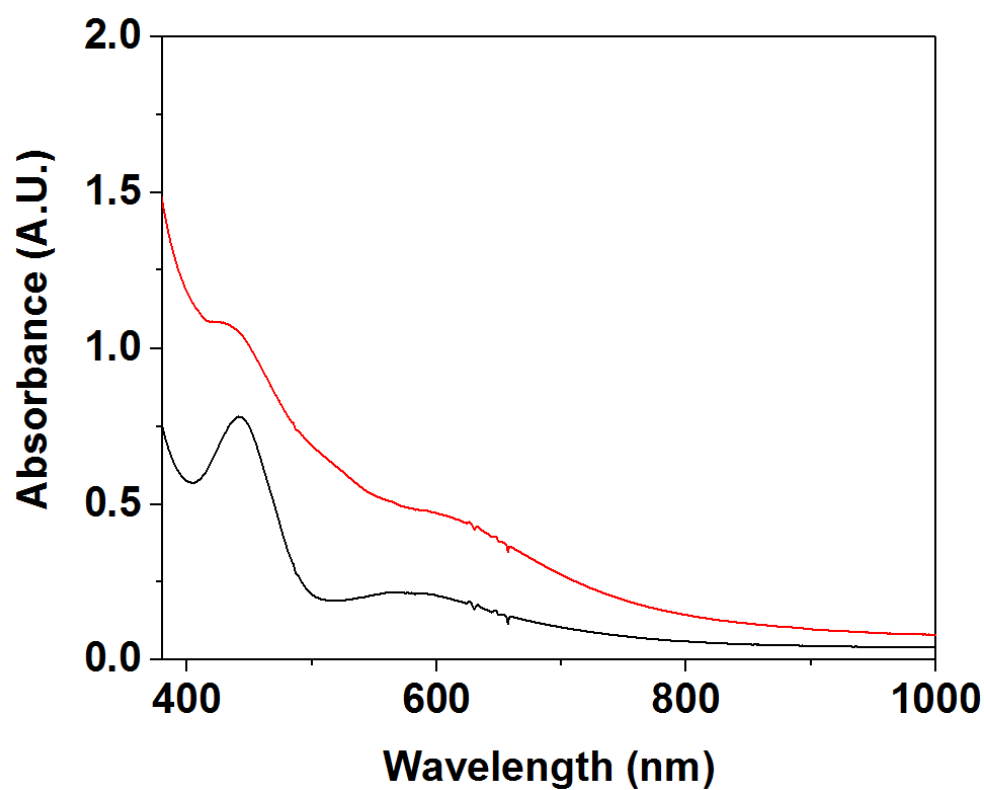


Figure A3.4: Electronic absorption spectra of 2 (black) + 1 eq. pTsOH (red) in 1:9 MeCN/THF at -90 °C.

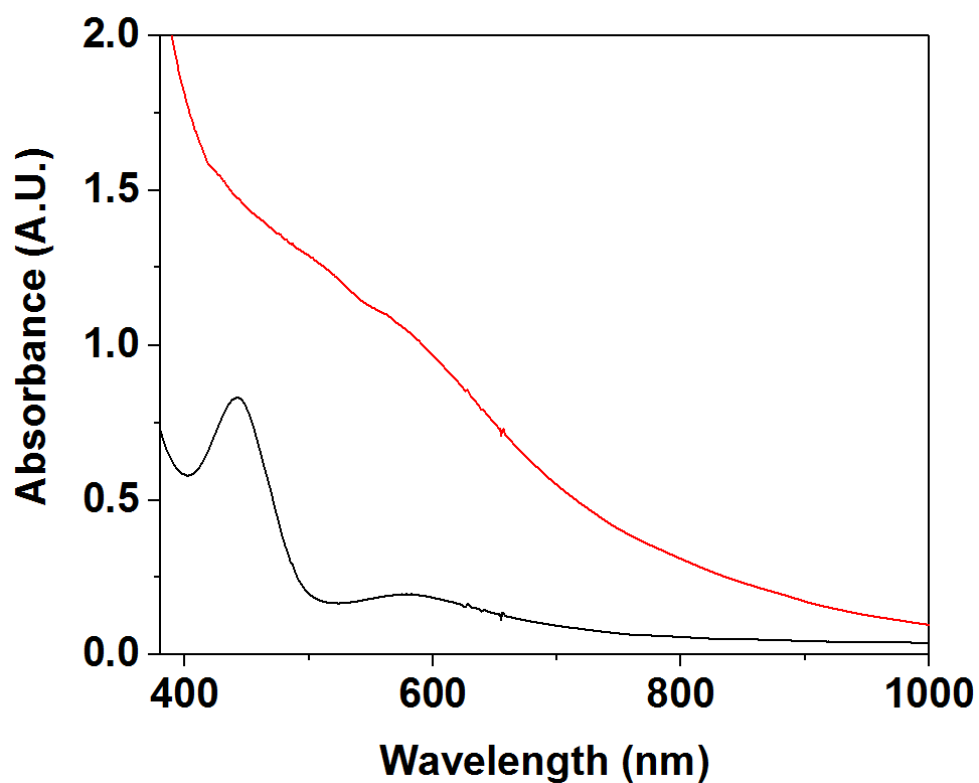


Figure A3.5: Electronic absorption spectra of **2** (black) + 1.5 eq. pTsOH (red) in 1:9 MeCN/THF at -90 °C.

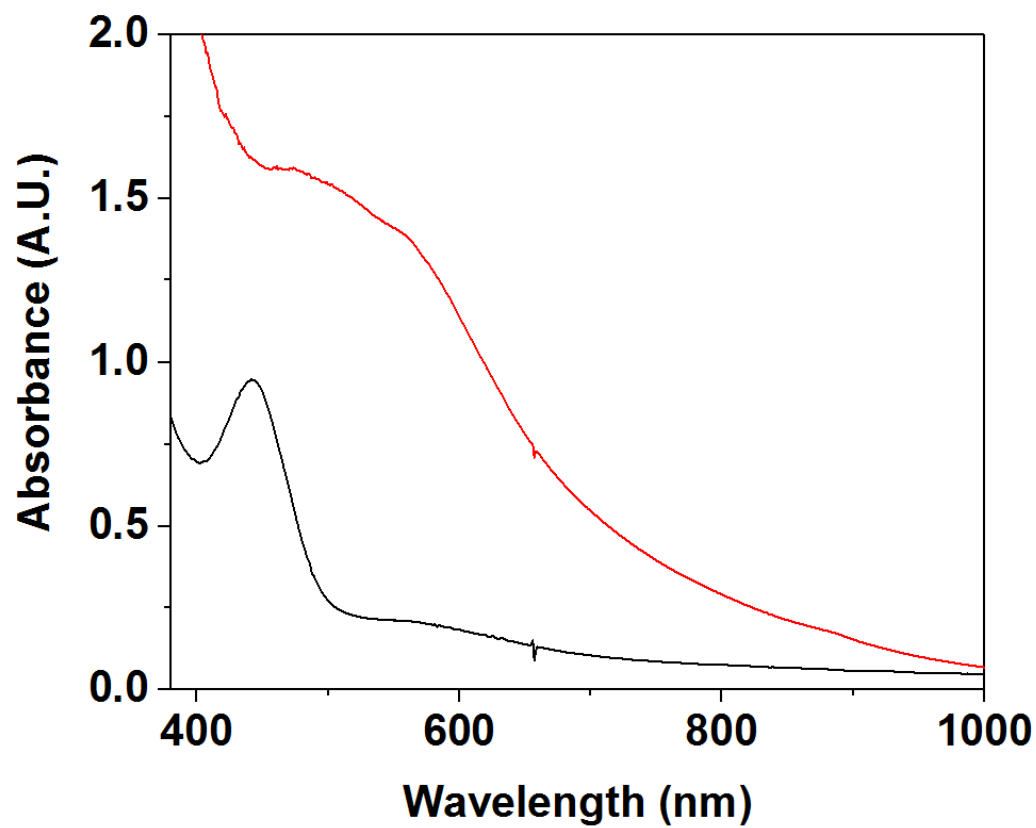


Figure A3.6: Electronic absorption spectra of **2** (black) + 2 eq. pTsOH (red) in 1:9 MeCN/THF at -90 °C.

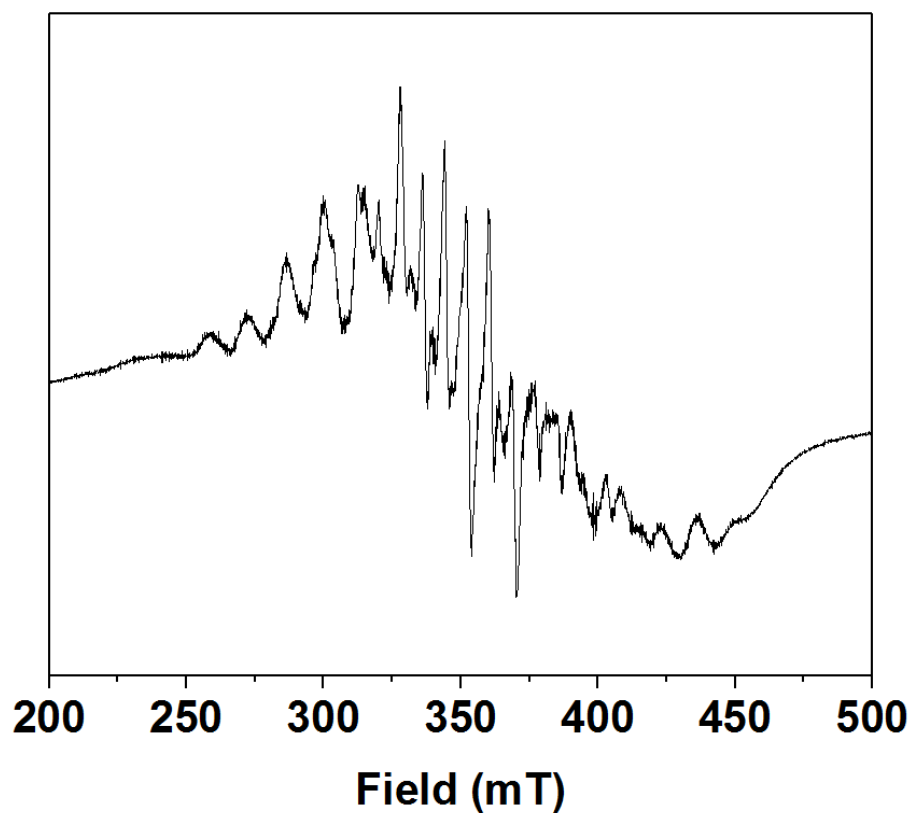


Figure A3.7: Perpendicular X-Band EPR spectrum of the post-reaction mixture of **2** + 1 eq. pTsOH measured at 2 K (9.65 GHz, 0.2 mW microwave power)

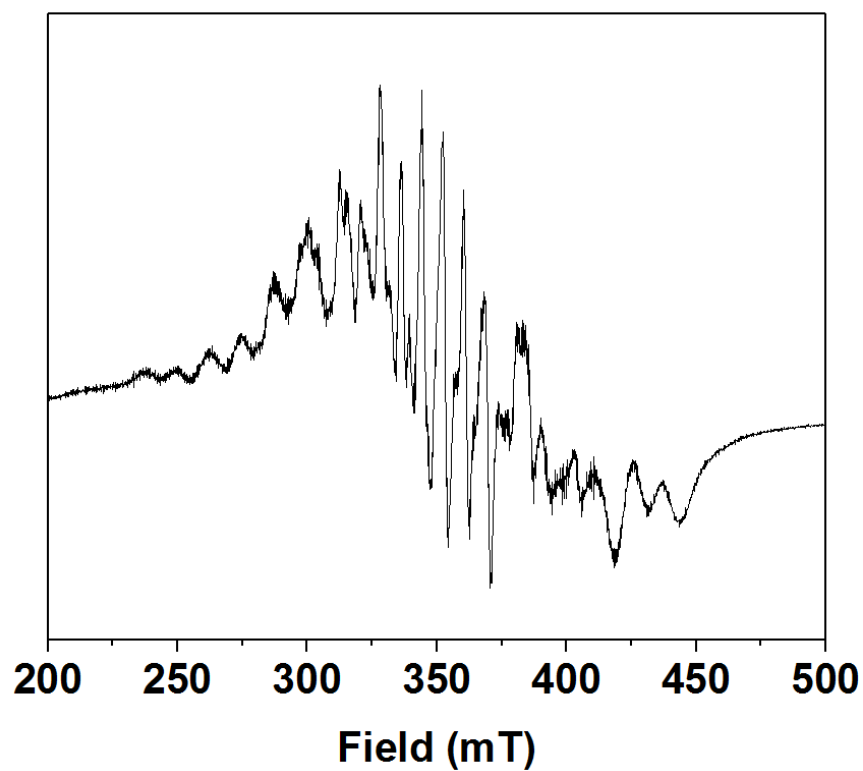


Figure A3.8: Perpendicular X-Band EPR spectrum of the post-reaction mixture of **2** + 1.5 eq. pTsOH measured at 2 K (9.65 GHz, 0.2 mW microwave power)

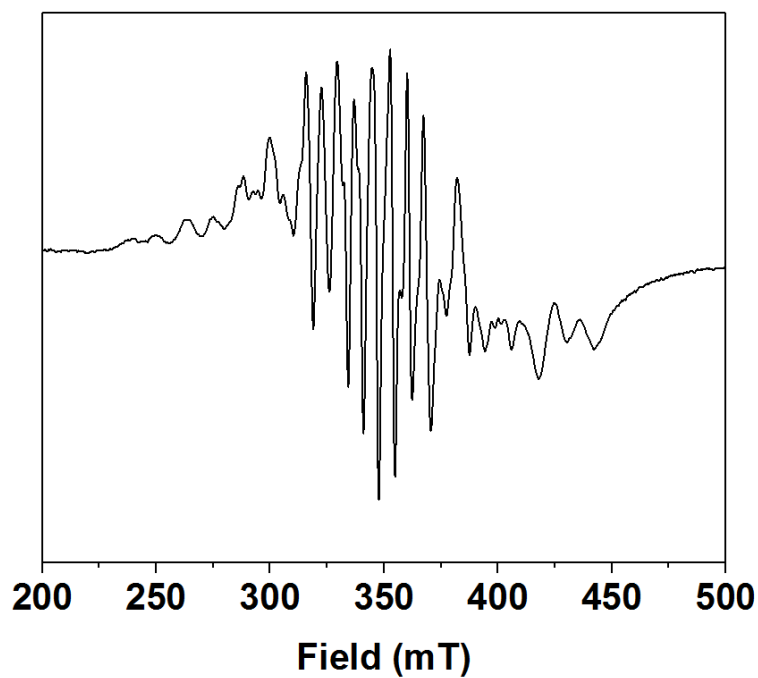


Figure A3.9: Perpendicular X-Band EPR spectrum of the post-reaction mixture of **2** + 2 eq. pTsOH measured at 2 K (9.65 GHz, 0.2 mW microwave power)

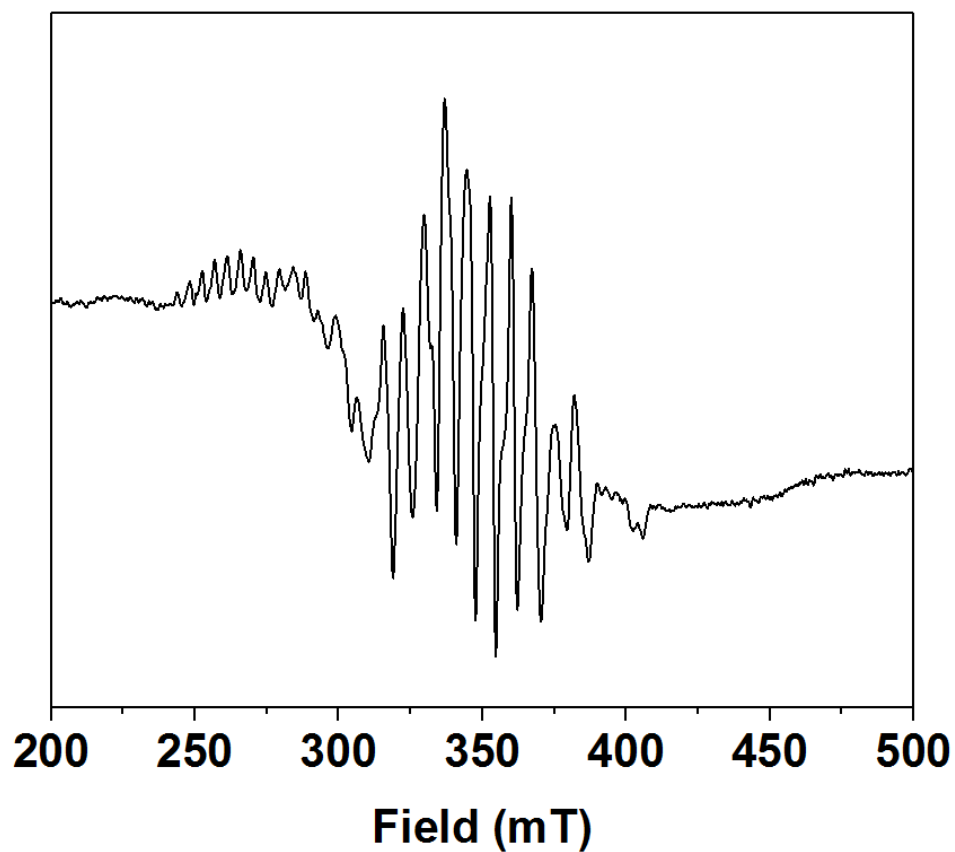


Figure A3.10: Perpendicular X-Band EPR spectrum of the post-reaction mixture of **2** + 2 eq. pTsOH measured at 30 K (9.65 GHz, 0.2 mW microwave power)

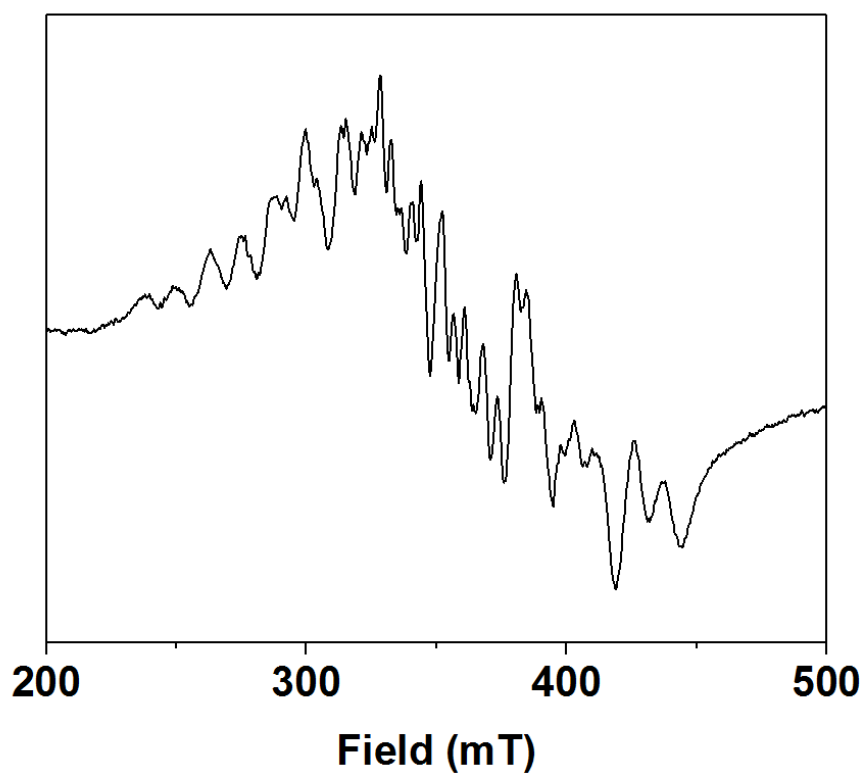


Figure A3.11: Perpendicular X-Band EPR spectrum of the post-reaction mixture of **2** + 3 eq. *pTsOH* measured at 30 K (9.65 GHz, 0.2 mW microwave power)

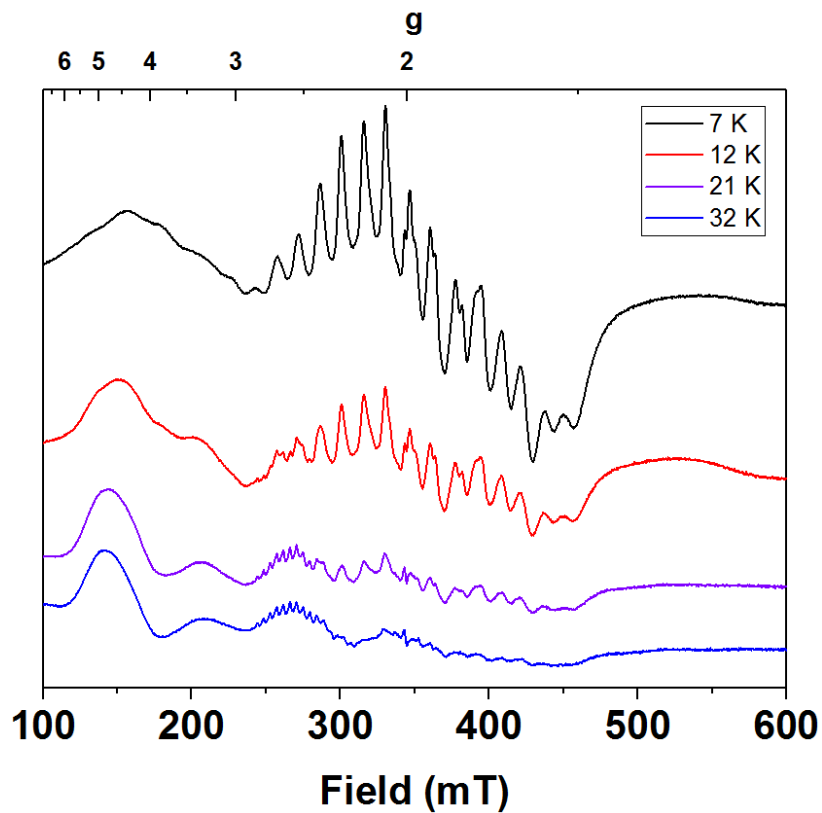


Figure A3.12: Perpendicular X-Band EPR spectrum of **2** (generated from 1.5 mM Mn_2BPMP + 1 eq. KO_2) measured at 7 K (black), 12 K (red), 21 K (purple), 32 K (blue) (9.65 GHz, 0.2 mW microwave power).

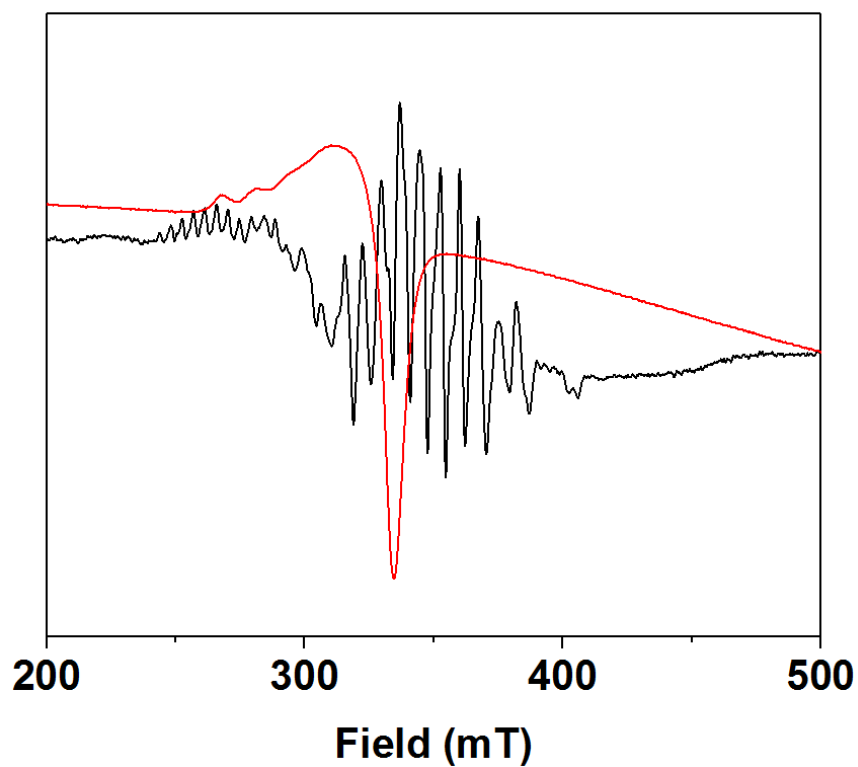


Figure A3.13: Perpendicular mode EPR spectra of **3** in 1:9 CH₃CN/THF (obtained from the reaction of 1.5 mM **1** with KO₂ in 1:9 CH₃CN/THF-black line) and Cu(NO₃)₂ standard (0.25 mM) in CH₃OH (red line) (30 K, 9.64 GHz, 0.2 mW microwave power).

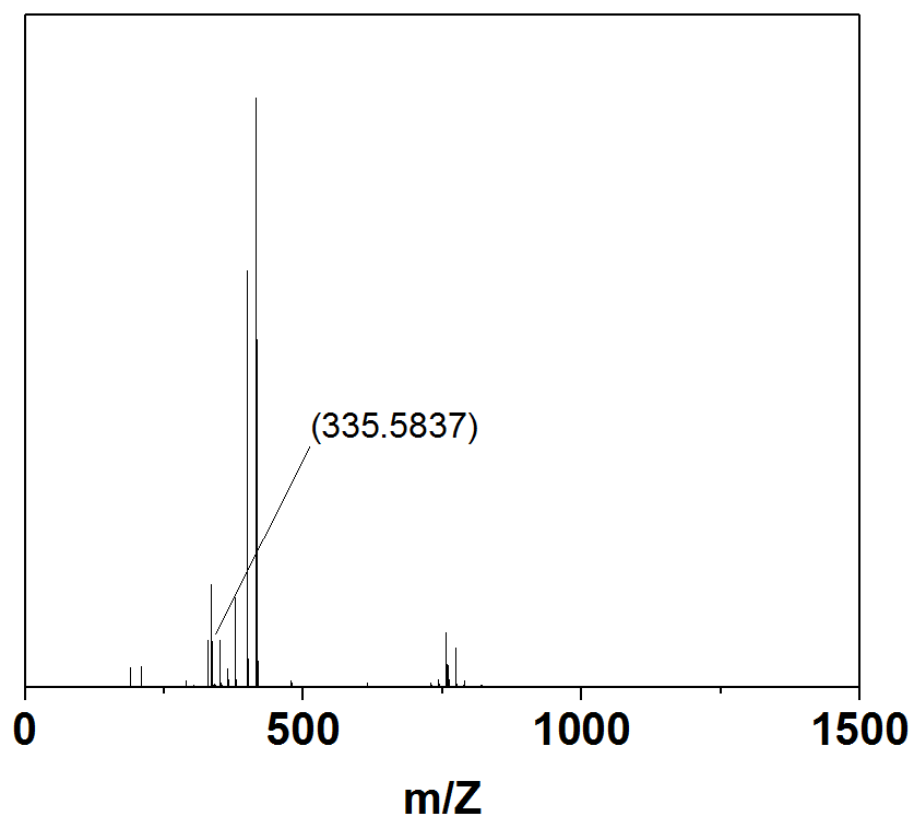


Figure A3.14: ESI-MS spectra of the post-reaction mixture of **2** + 2 eq. pTsOH. Highlighted peak corresponds to **4**.

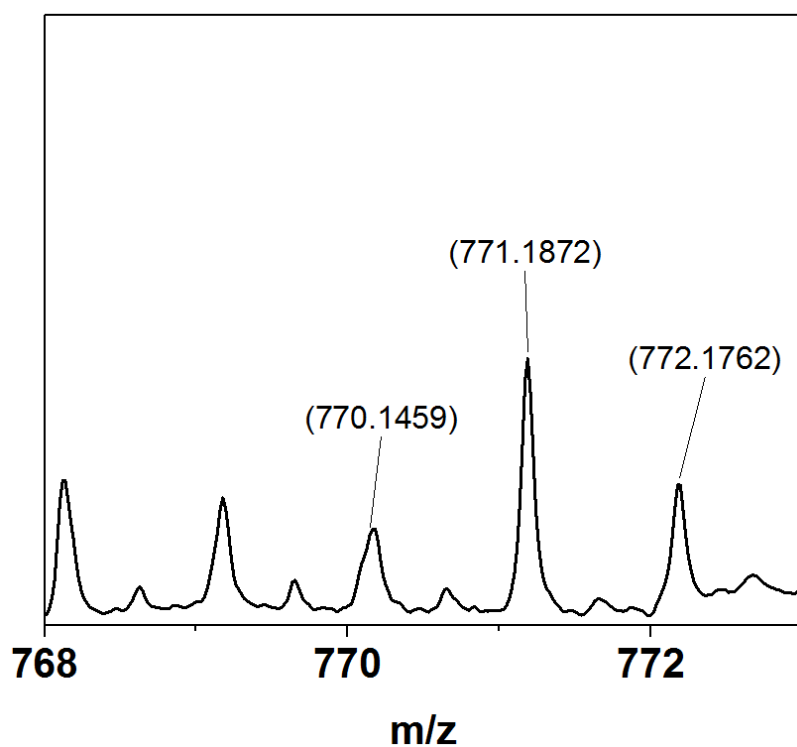


Figure A3.15: ESI-MS spectra of the post-reaction mixture of **4** expanded in the region where **2** would be expected. (**2** m/z = 770.29).

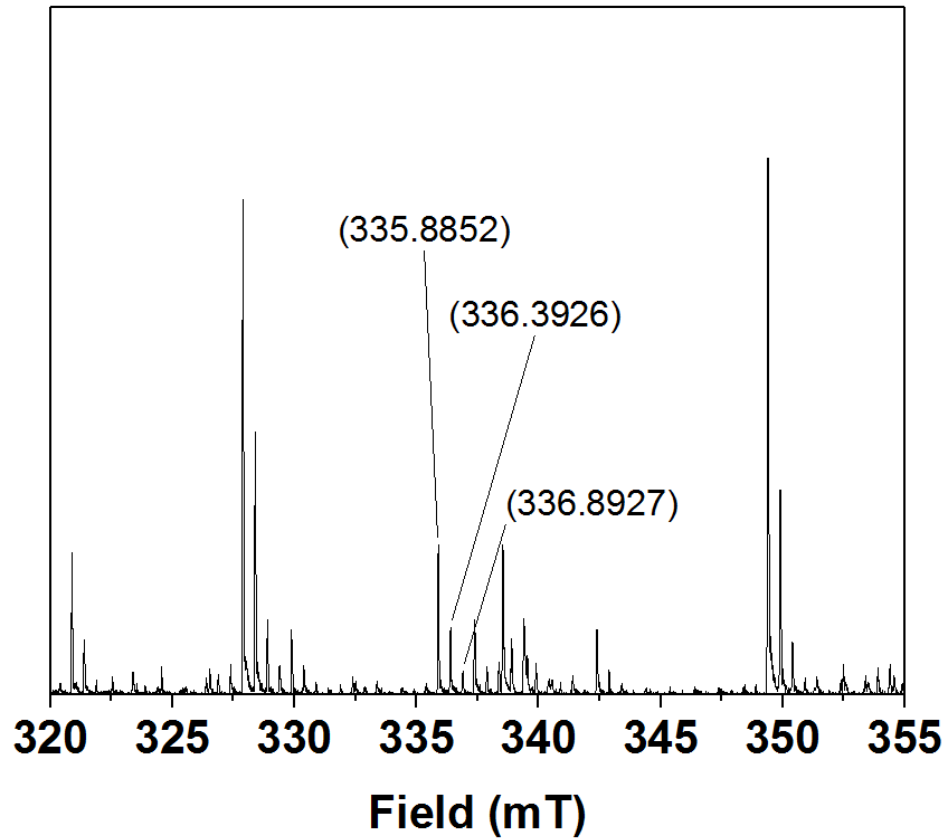


Figure A3.16: ESI-MS spectra of the post-reaction mixture of **2** expanded in the region where **4** would be expected. (**4** m/z = 335.5682)

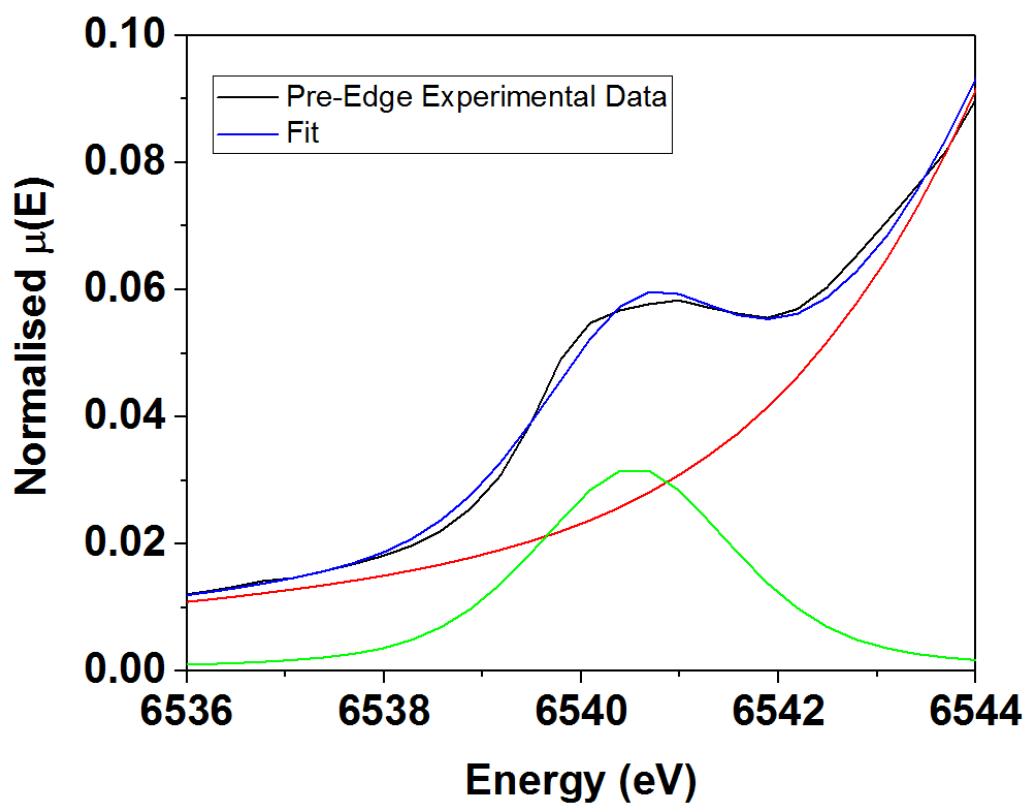


Figure A3.17: Rising edge and peak fitting of the pre-edge area of 4 with a 50:50 pseudo-Voigt function.

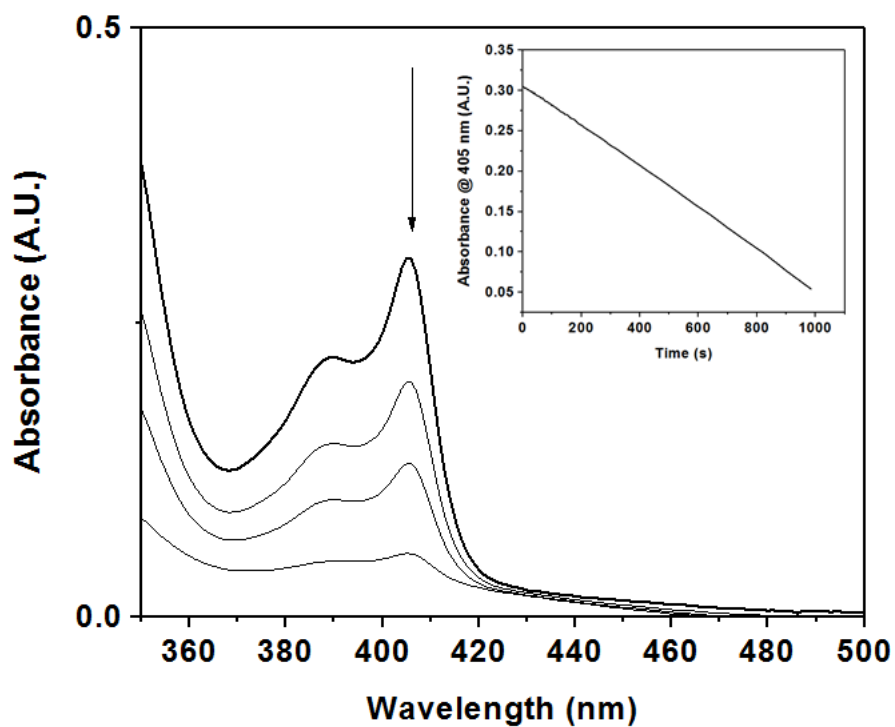


Figure A3.18: The decay of the electronic absorption features of 0.8 mM 4-methoxy-2,6-di-tert-butylphenoxyl radical in 1:9 MeCN/THF at 20 °C. Inset: Decay of the feature at $\lambda_{nm} = 405$ nm over time.

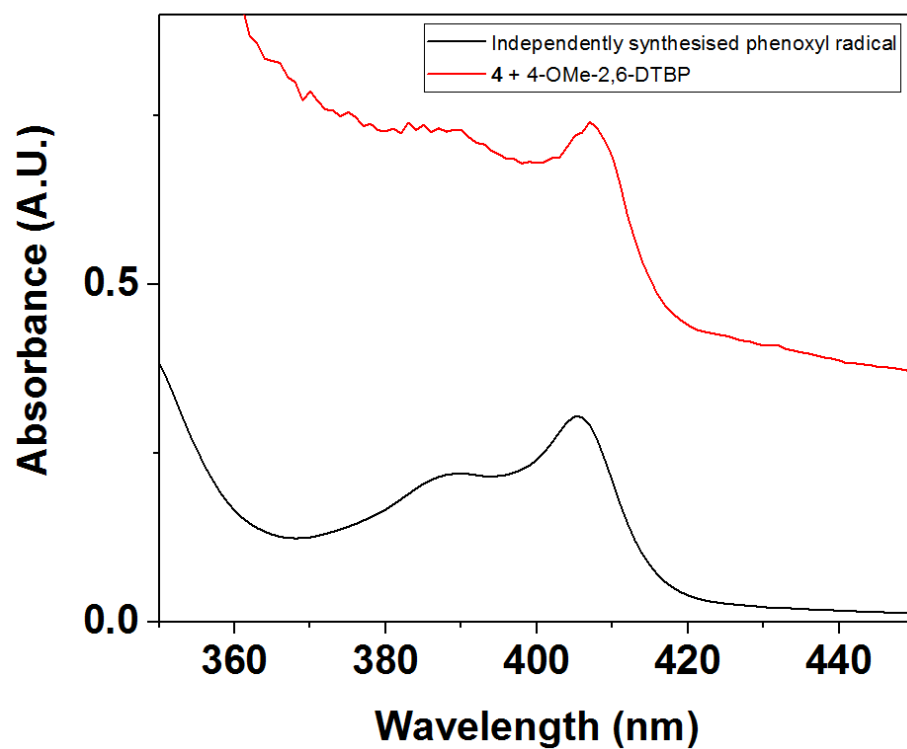


Figure A3.19: The electronic absorption spectra of the independently synthesised 4-OCH₃-2,6-di-*tert*-butylphenoxyl radical (black). The electronic absorption spectra of the reaction of 4 with 4-OCH₃-2,6-DTBP (red)

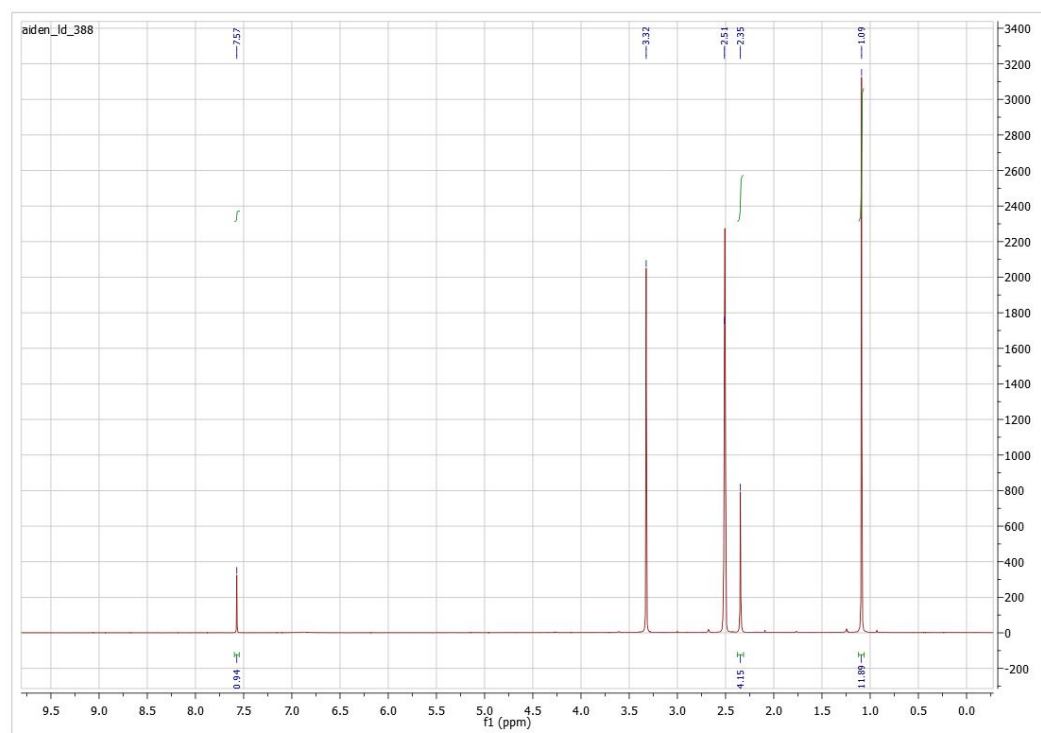


Figure A3.20: NMR spectra of 4-oxo-TEMPO-H.

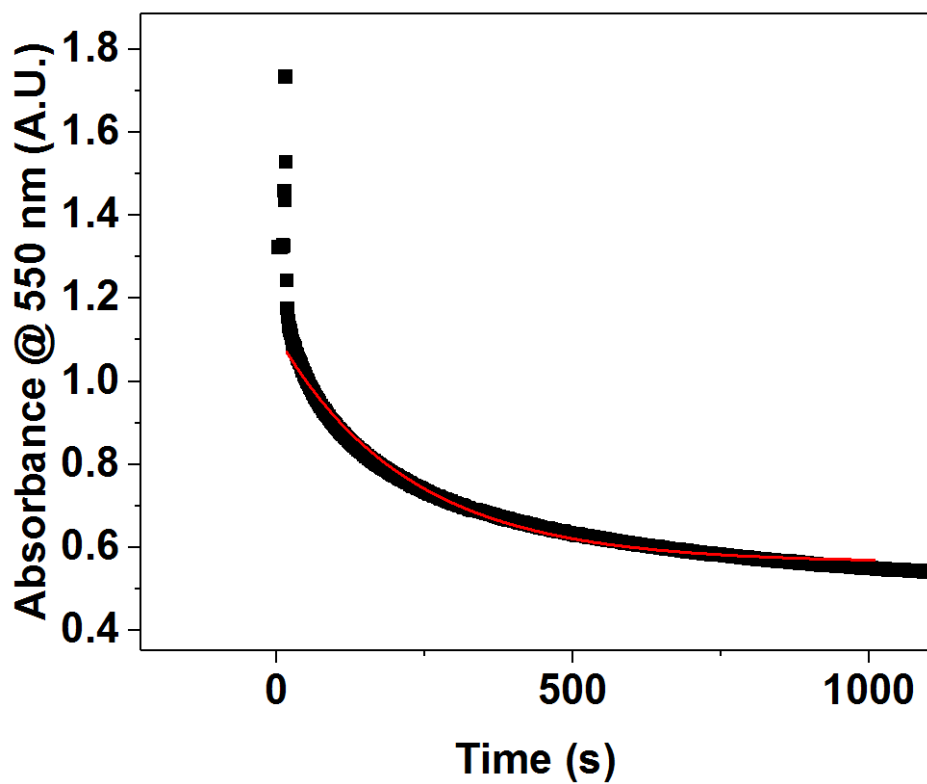


Figure A3.21: Plot of the absorbance at 550 nm during the reaction of **4** and 4-oxo-TEMPO-H (black trace). Pseudo first-order fit of the resulting decay (red trace).

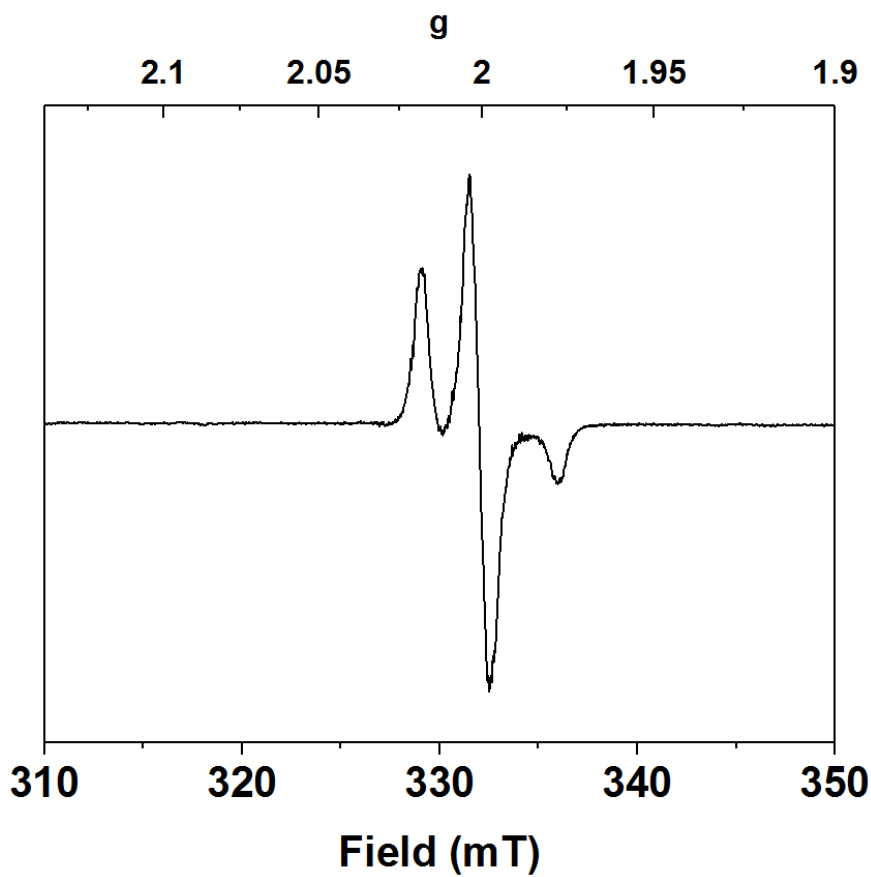


Figure A3.22: EPR Spectra of the post-reaction mixture of **4** and 4-oxo-TEMPO-H

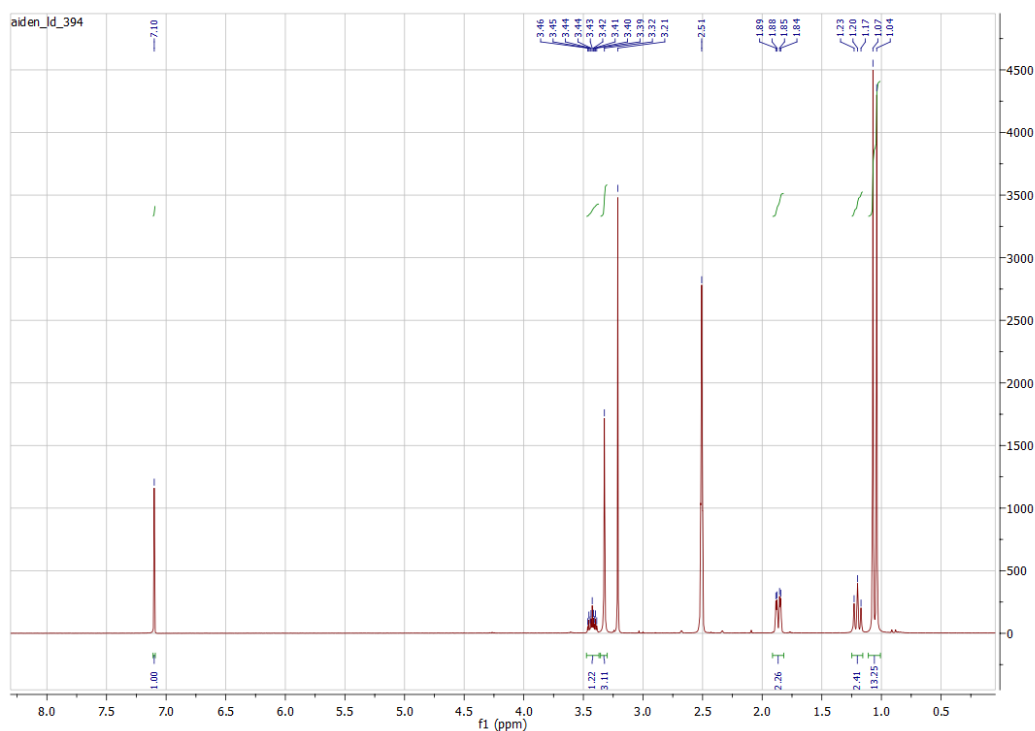


Figure A3.23: NMR spectra of 4-OCH₃-TEMPO-H

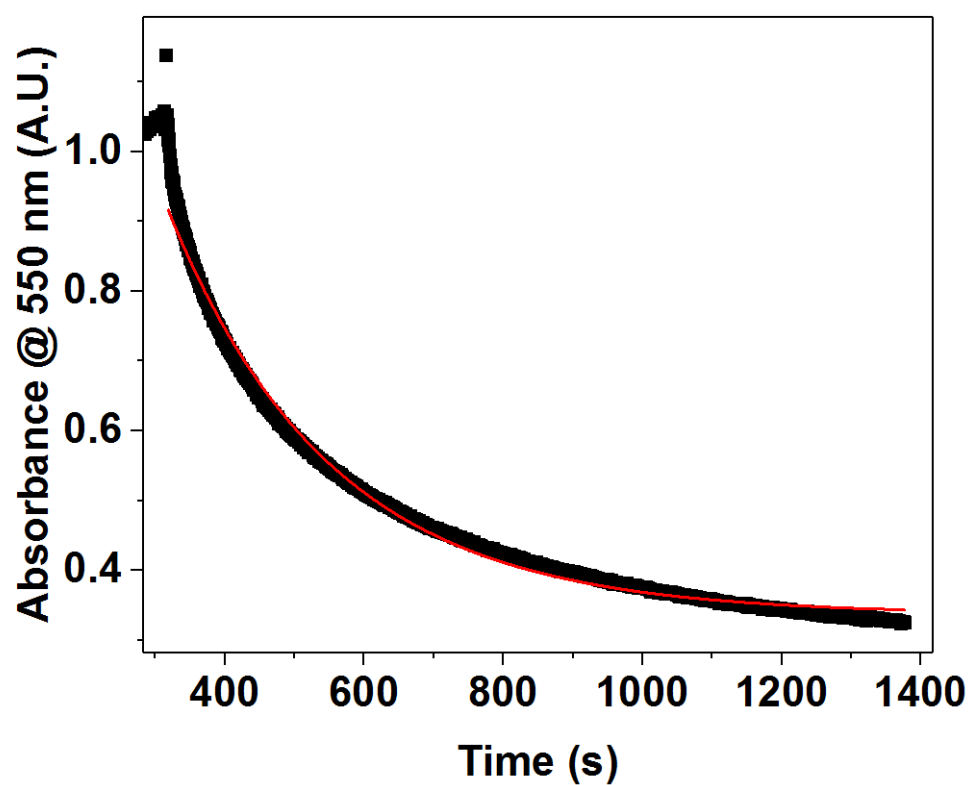


Figure A3.24: Plot of the absorbance at 550 nm during the reaction of 4 with 4-OCH₃-TEMPO-H (black trace). Pseudo first-order fit of the resulting decay (red trace).

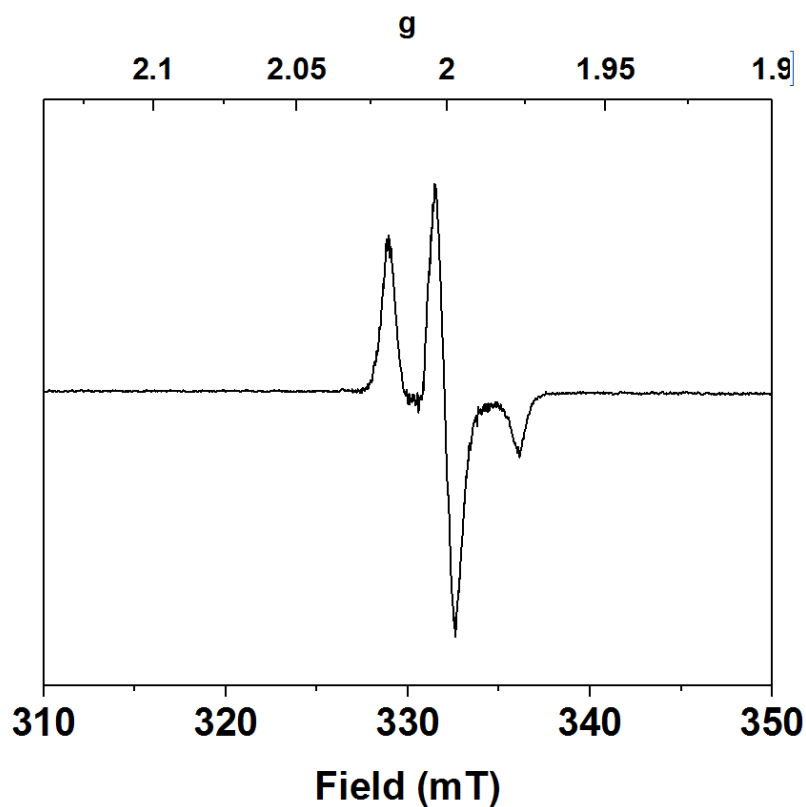


Figure A3.25: EPR spectra of the post-reaction mixture of 4 and 4-OCH₃-TEMPO-H

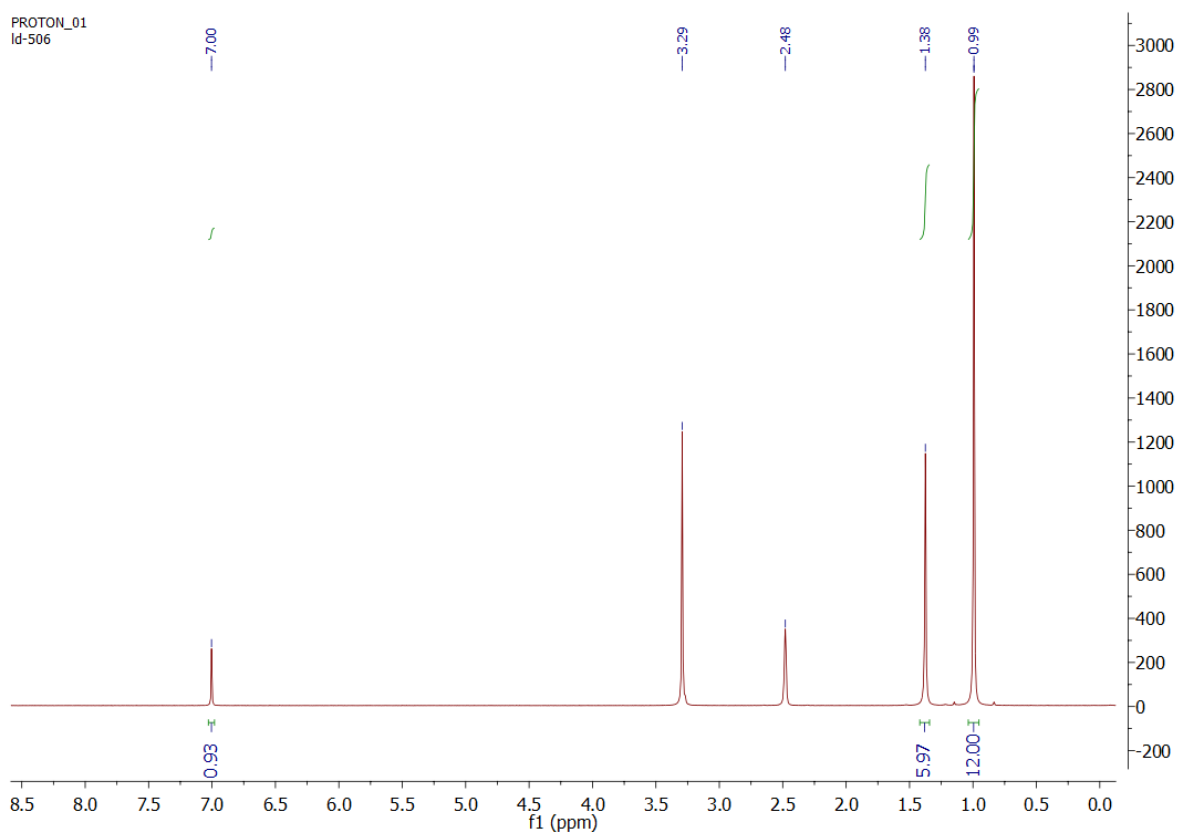


Figure A3.26: NMR spectra of TEMPO-H

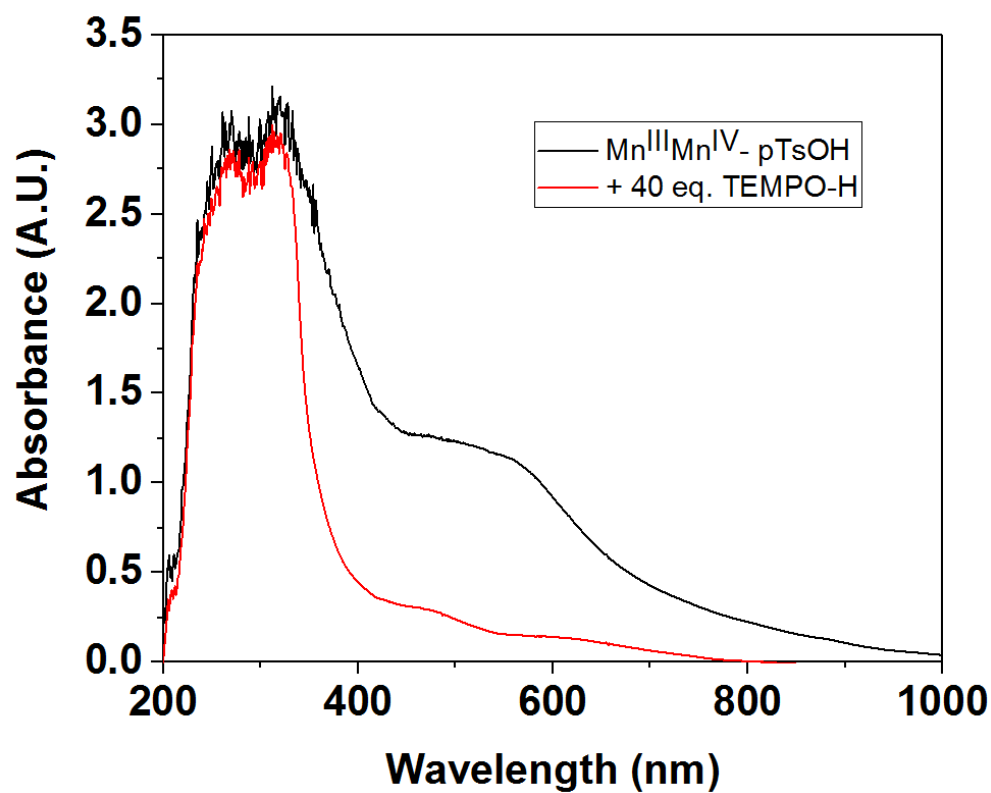


Figure A3.27: Electronic absorption spectra of the reaction between **4** (black trace) and 40 eq. TEMPO-H (red trace).

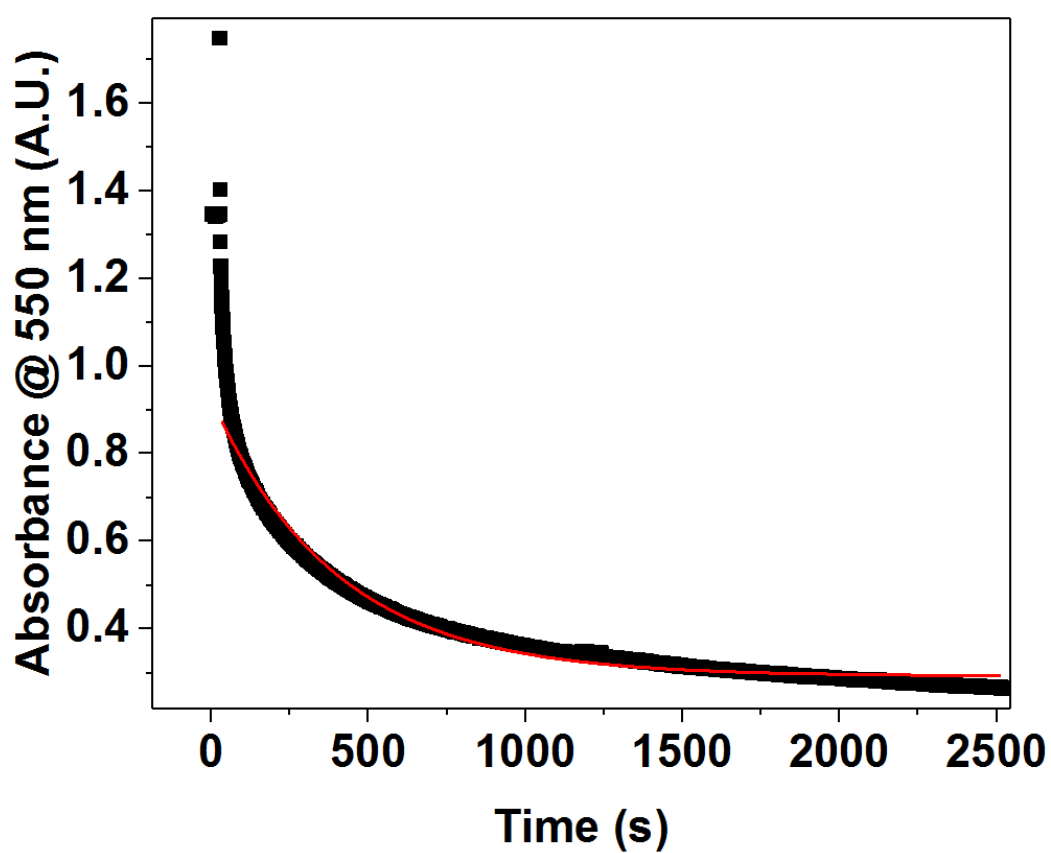


Figure A3.28: Plot of the absorbance at 550 nm during the reaction of **4** and TEMPO-H (black trace). Pseudo first-order fit of the decay (red trace)

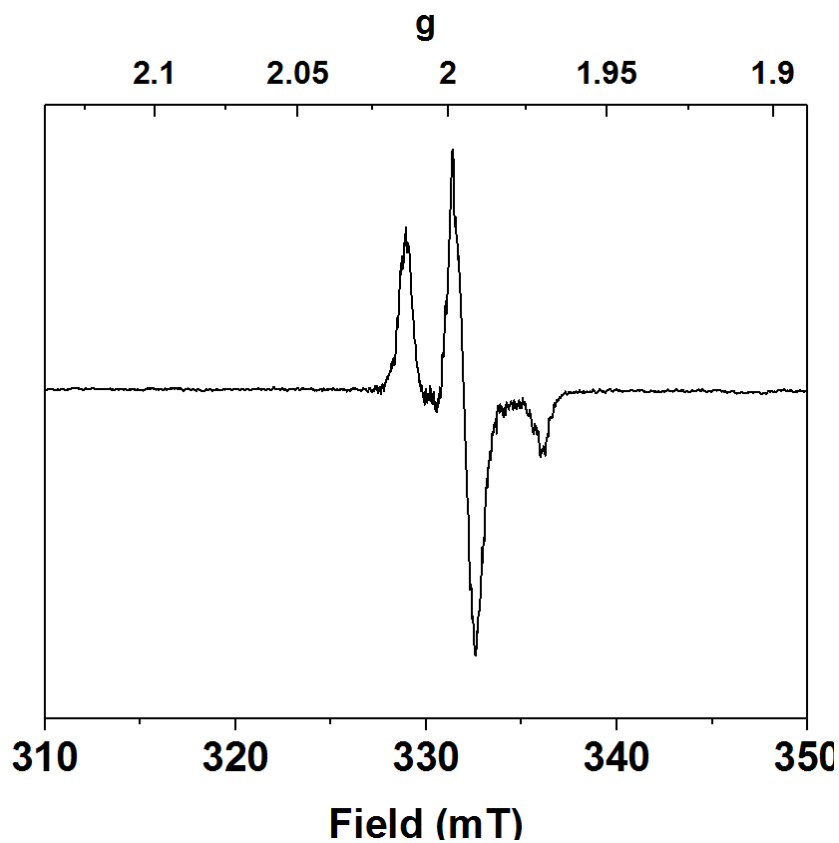


Figure A3.29: EPR spectra of the post-reaction mixture of 4 and TEMPO-H

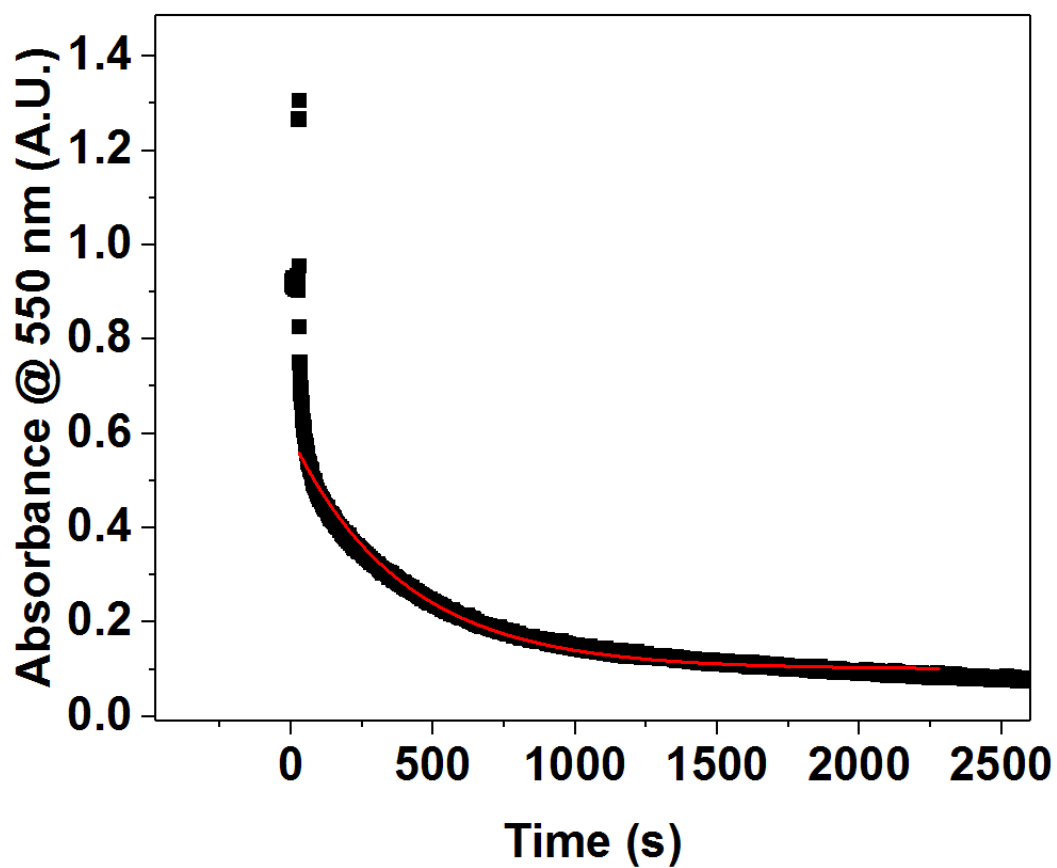


Figure A3.30: Plot of absorbance at 550 nm during the reaction of 4 and 4-OCH₃-2,6-DTBP (black trace). Pseudo first order fit of the resulting decay (red trace).

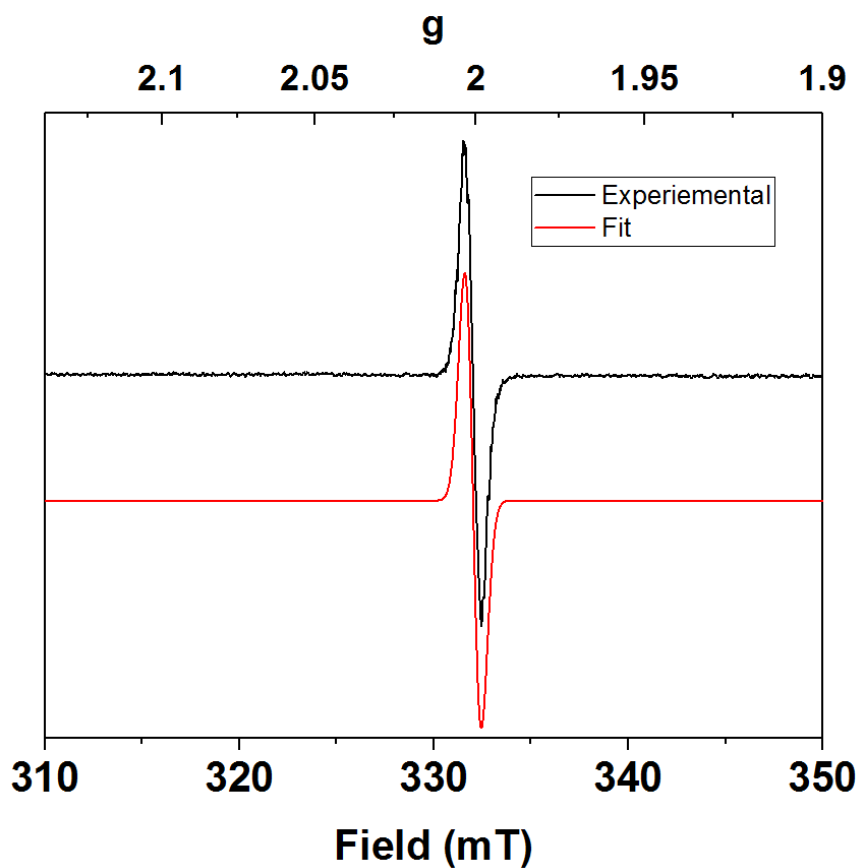


Figure A3.31: EPR spectra of the post-reaction mixture of 4 and 4-OCH₃-2,6-di-tert-butylphenol.

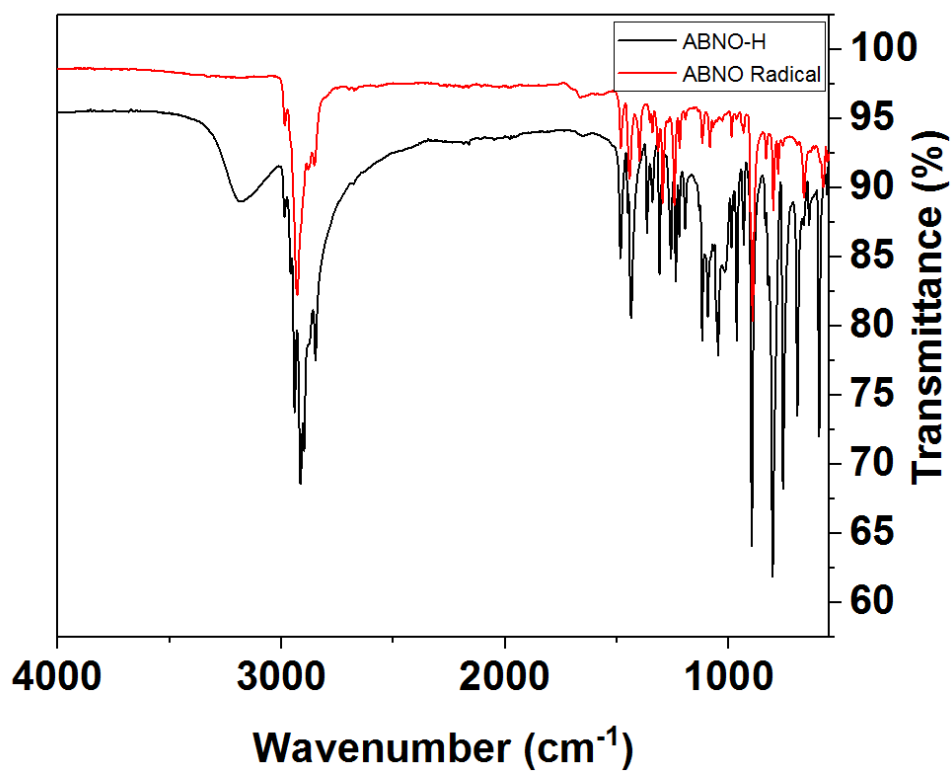


Figure A3.32: IR spectra of ABNO-H (black trace) and ABNO radical (red trace).

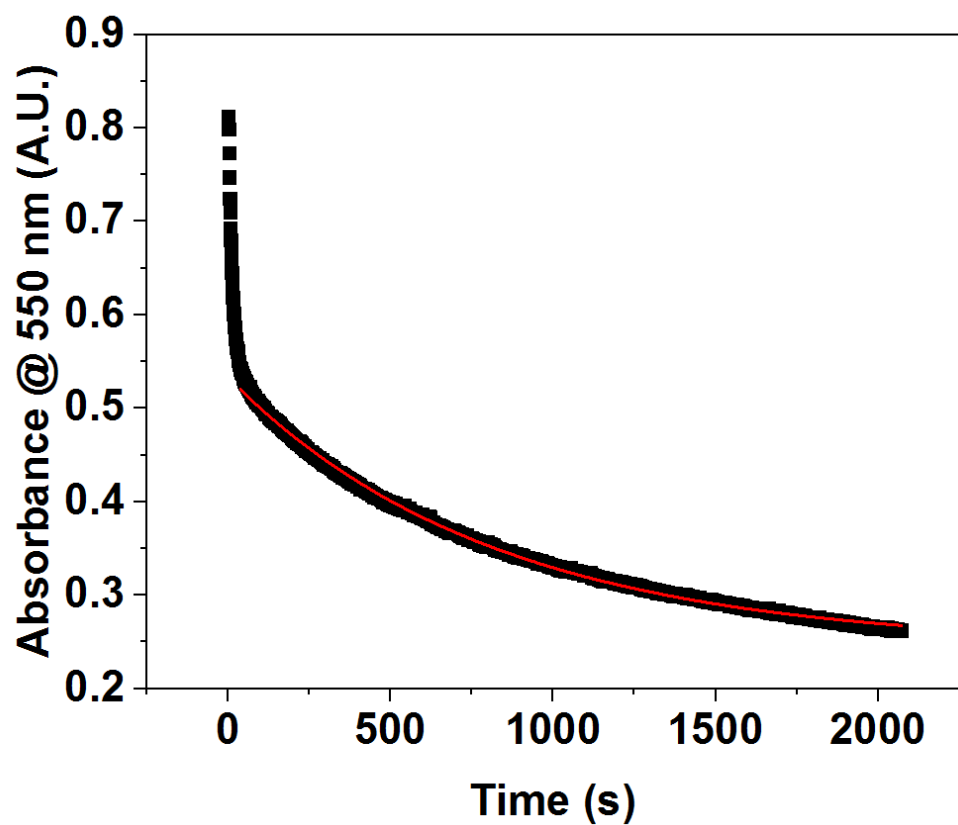


Figure A3.33: Plot of absorbance at 550 nm during the reaction of **4** and ABNO-H (black trace). Pseudo first order fit of the resulting decay (red trace).

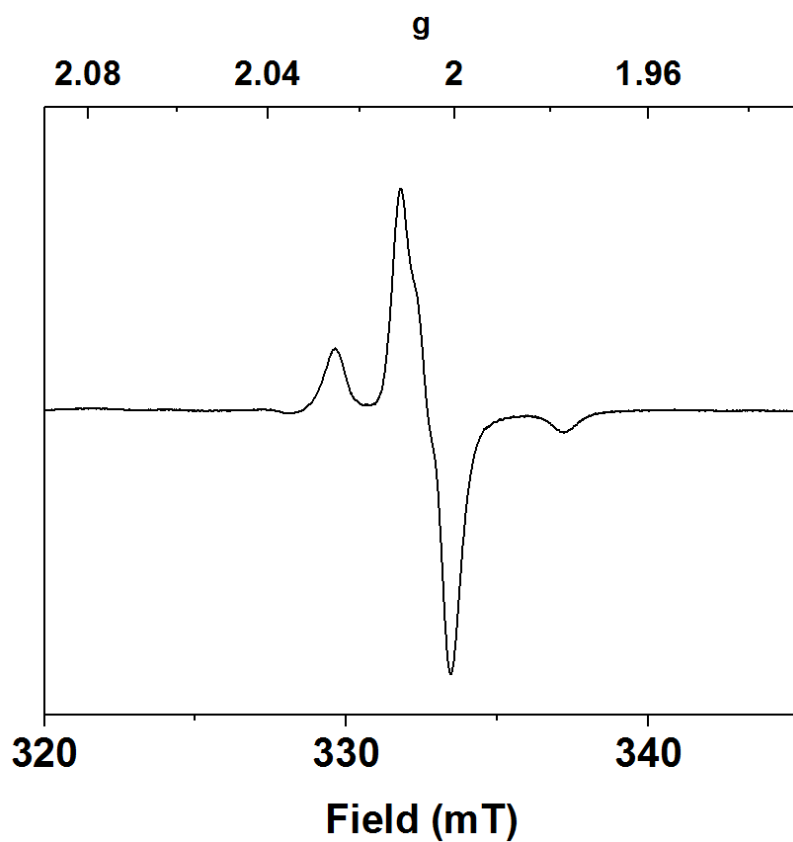


Figure A3.34: EPR spectra of the post-reaction mixture of **4** and ABNO-H.

Substrate	EPR Radical Yield
TEMPO-H	75 %
4-OCH ₃ -TEMPO-H	60 %
4-Oxo-TEMPO-H	75 %
ABNO-H	40 %
4-OCH ₃ -2,6-DTPB	35 %

Figure A3.35: Yields of the readical species generated after reaction with **4** determined by EPR spectroscopy.

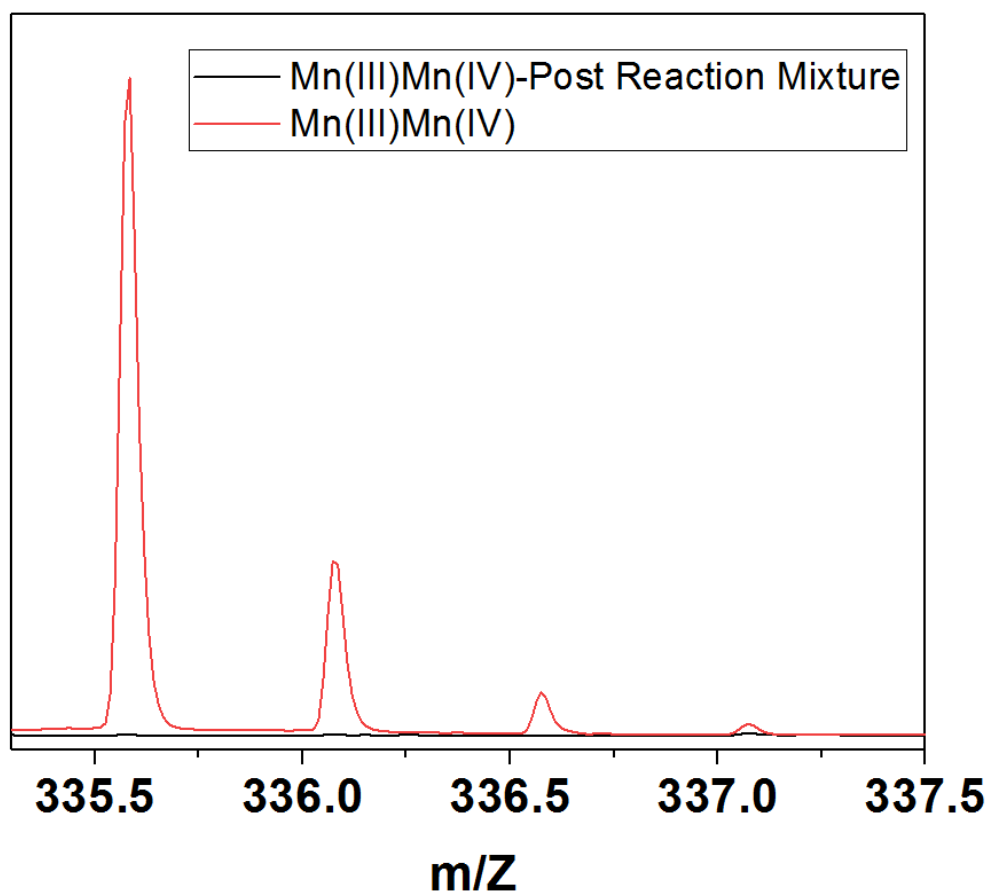


Figure A3.36: ESI-MS spectra of the post-reaction mixture of **4** and TEMPO-H (black). ESI-MS spectra of **4** (red).

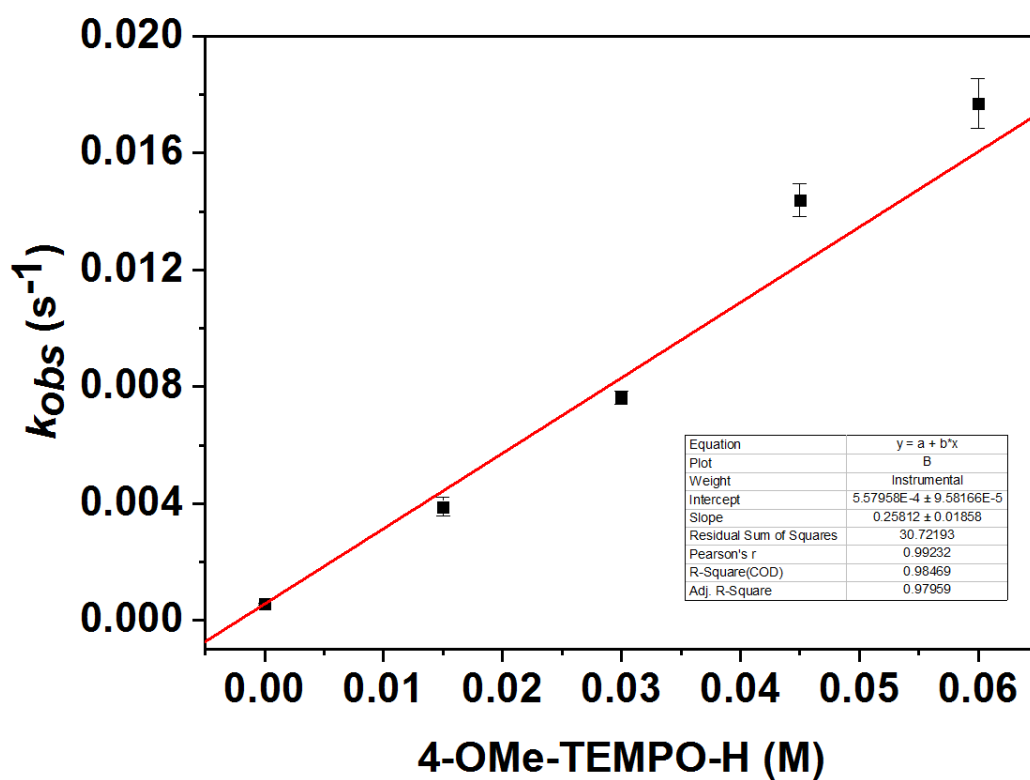


Figure A3.37: Plot of k_{obs} versus $[4\text{-OCH}_3\text{-TEMPO-H}]$ determined for the reaction of **4** and 4-OCH₃-TEMPO-H

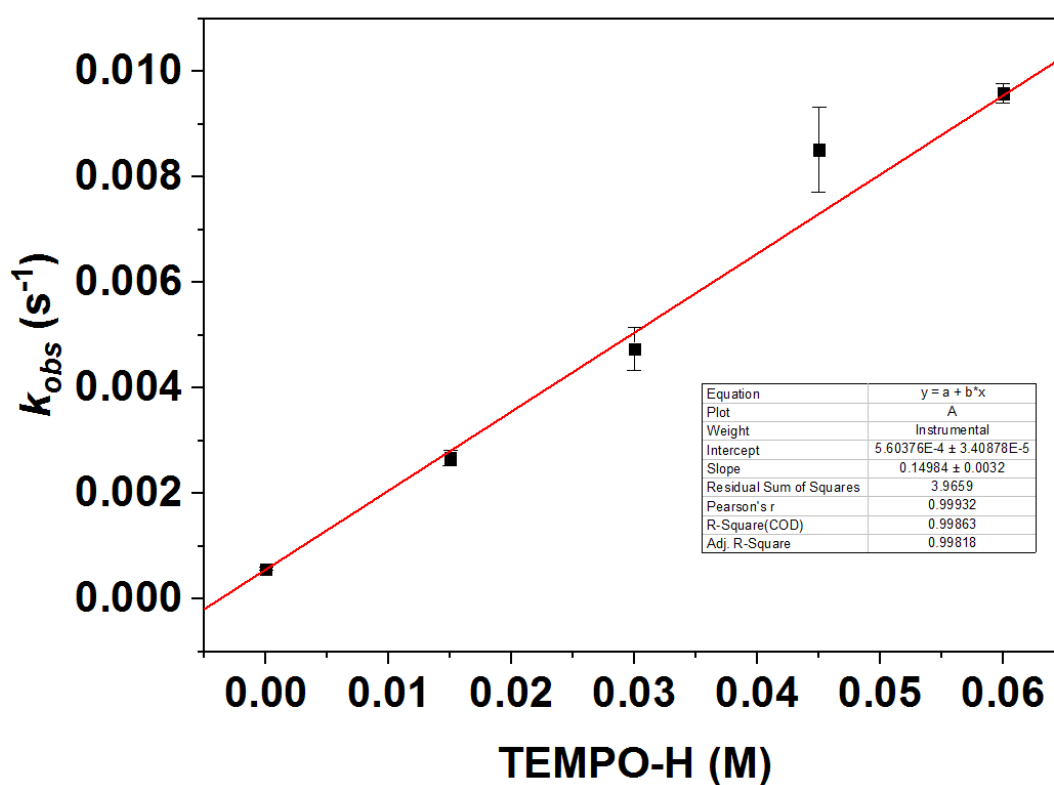


Figure A3.38: Plot of k_{obs} versus $[\text{TEMPO-H}]$ determined for the reaction of **4** and TEMPO-H.

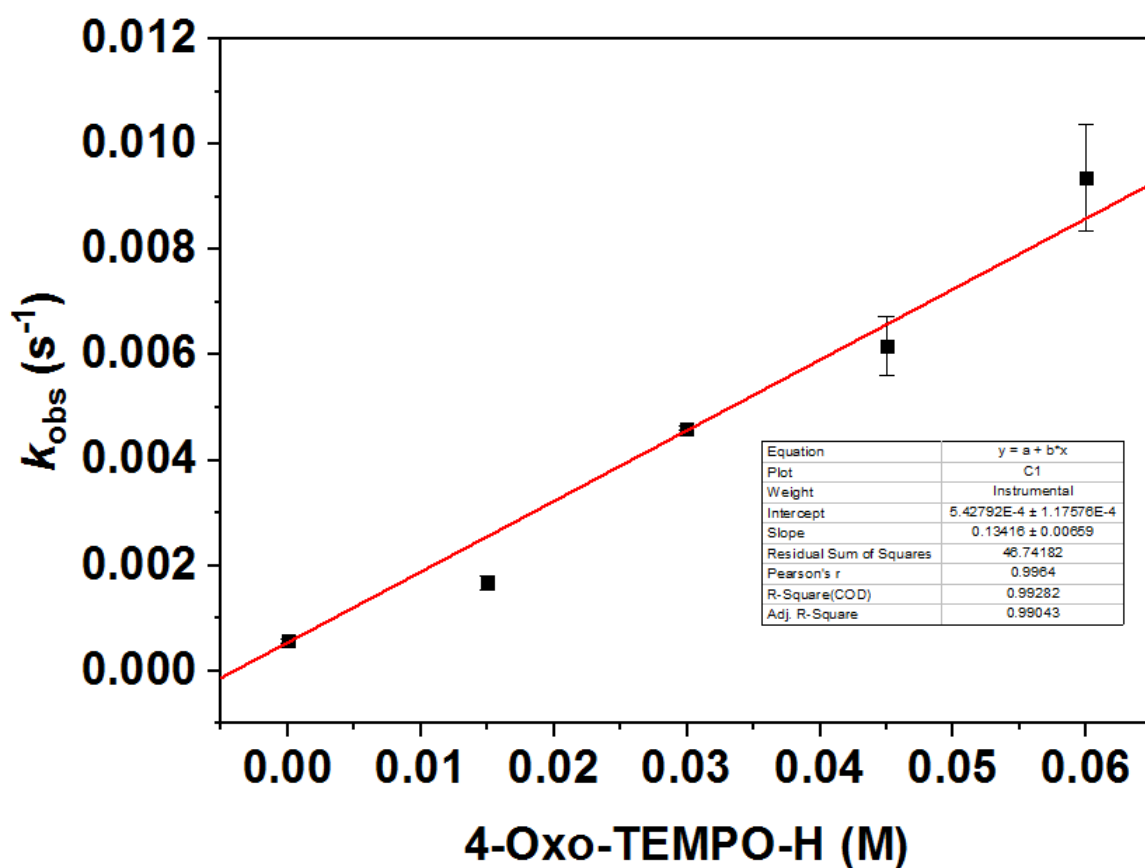


Figure A3.39: Plot of k_{obs} versus [4-Oxo-TEMPO-H] determined for the reaction 4 and 4-Oxo-TEMPO-H.

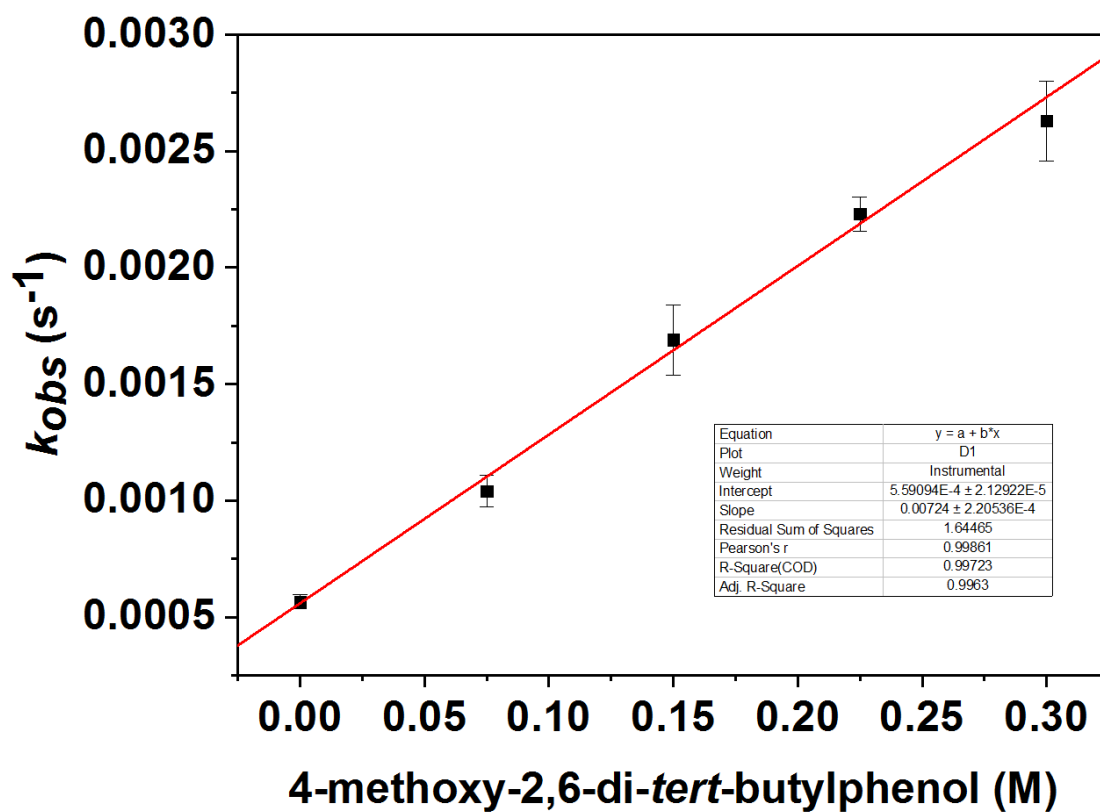


Figure A3.40: Plot of k_{obs} versus [4-OCH₃-2,6-DTBP] determined for the reaction of 4 and 4-OCH₃-2,6-DTBP

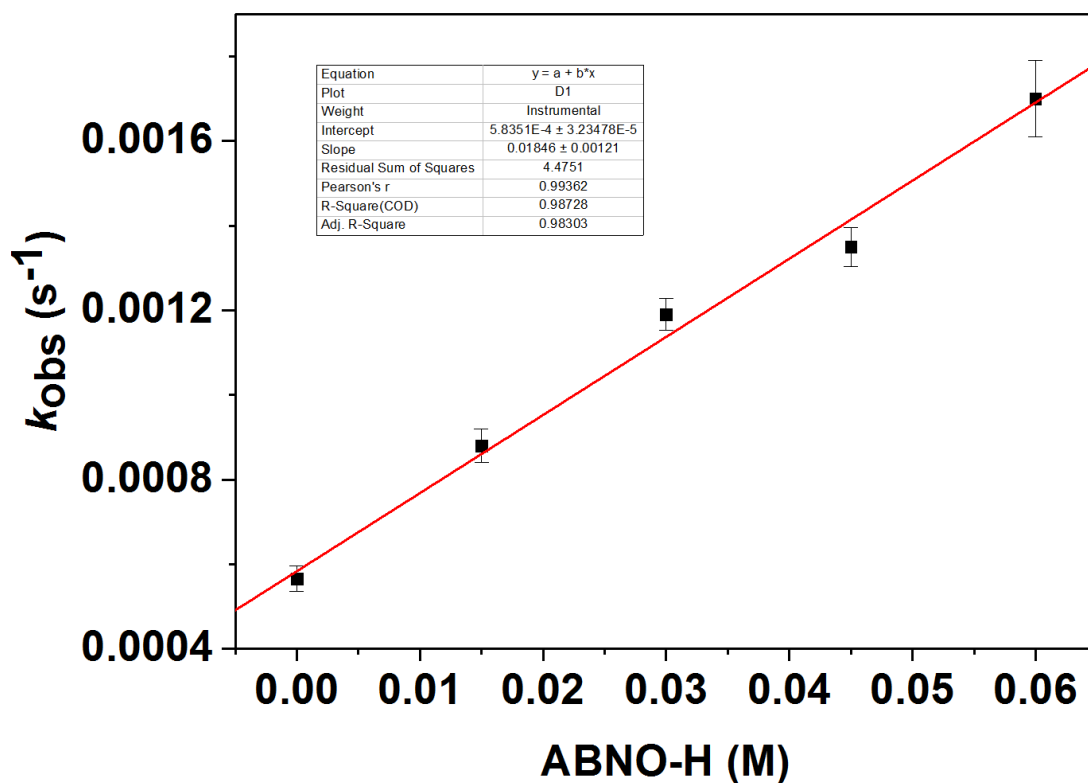


Figure A3.41: Plot of k_{obs} versus $[ABNO-H]$ determined for the reaction of **4** and ABNO-H

Calculation of ABNO-H BDFE in MeCN^{62,63}:

$$BDFE_g = BDE_g - TS^\circ(H^\circ)$$

$$ABNO\ BDE_g = 68.2\ kcal\ mol^{-1}$$

$$S^\circ(H^\circ) = 27.42\ cal\ K^{-1}mol^{-1}$$

$$BDFE_g = 63.179\ kcal\ mol^{-1}$$

$$BDFE_{solv} = BDFE_g + \Delta G^\circ_{solv}(H^\circ) + [\Delta G^\circ(X^\circ) - \Delta G^\circ(XH)]$$

$$\Delta G^\circ_{solv}(H^\circ) = 5.11\ kcal\ mol^{-1}\ in\ MeCN$$

$$\Delta G^\circ = -10.02\alpha_2^H\beta_2^H - 1.492$$

$$\alpha_2^H = 0.39\ (TEMPO - H)\ \beta_2^H = 0.44\ (MeCN)$$

$$\Delta G^\circ = 3.21$$

$$ABNO - H\ BDFE_{MeCN} = 71.49\ kcal\ mol^{-1}$$

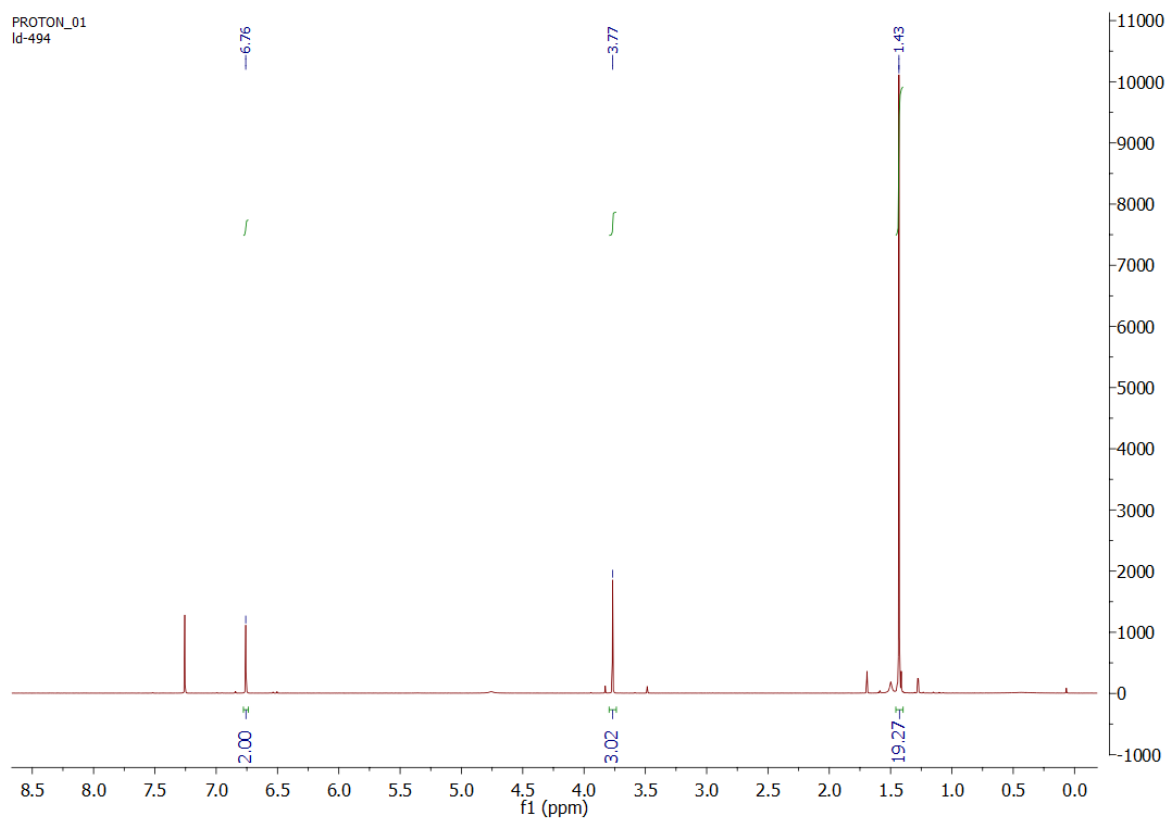


Figure A3.42: NMR spectra of OD-4-OCH₃-2,6-DTBP.

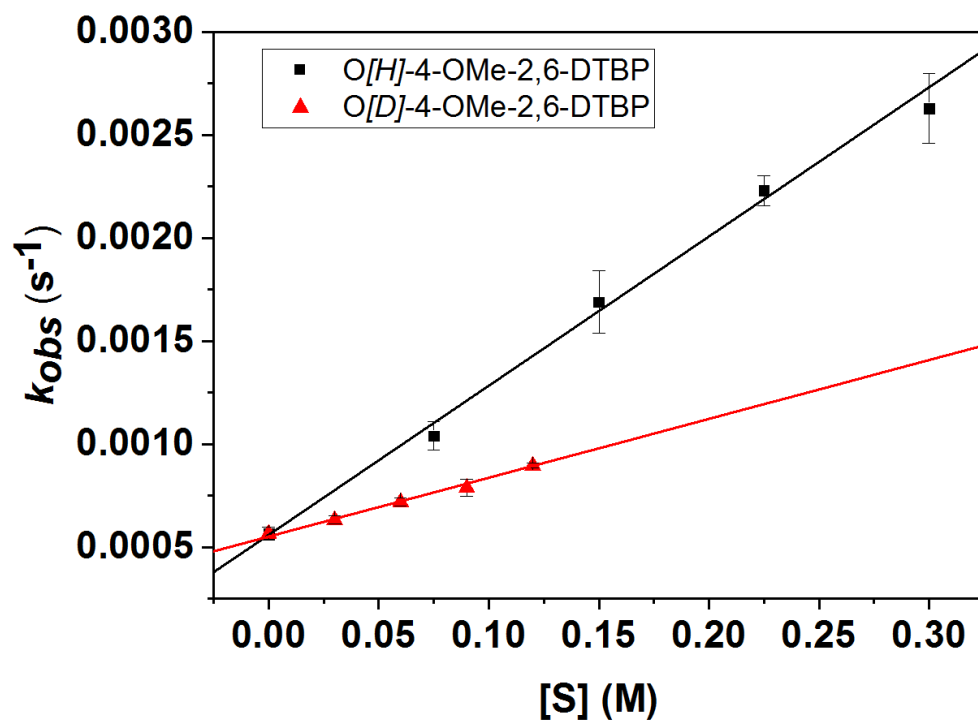


Figure A3.43: Plot of k_{obs} versus substrate determined for the reaction between complex 3 and 4-proto-OCH₃-2,6-DTBP (black trace) and 4-deutero-OCH₃-2,6-DTBP (red trace).

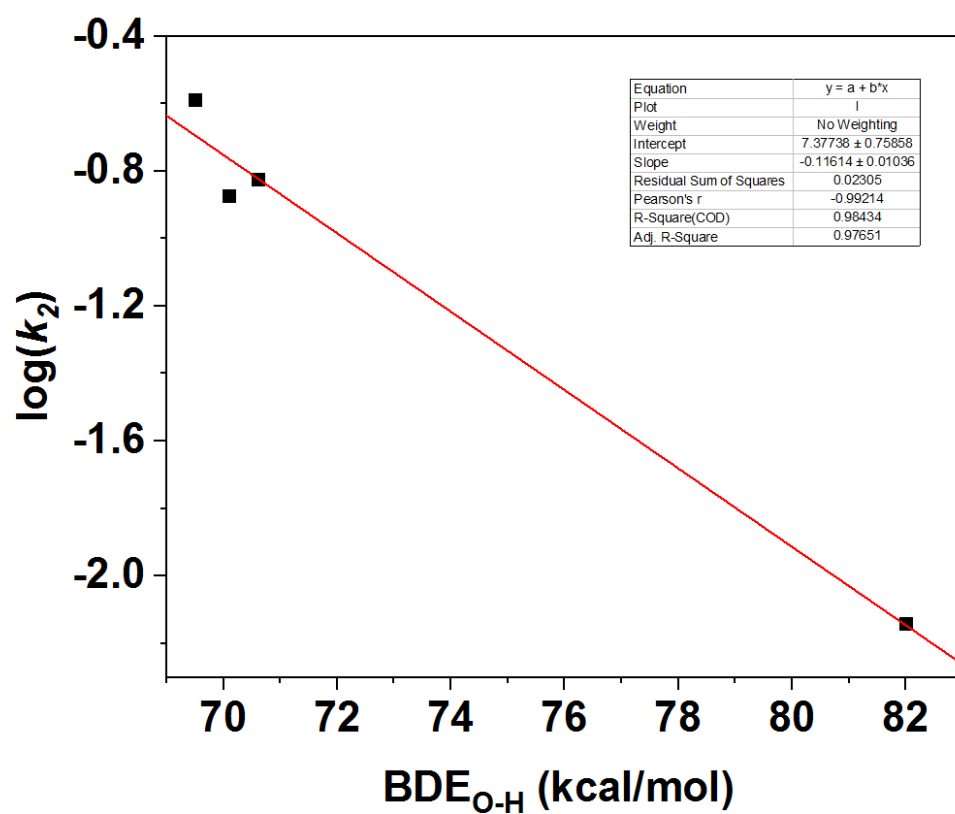


Figure A3.44: Plot of $\log(k_2)$ versus BDE_{O-H} for the phenol substrates.

Chapter 4 Appendix

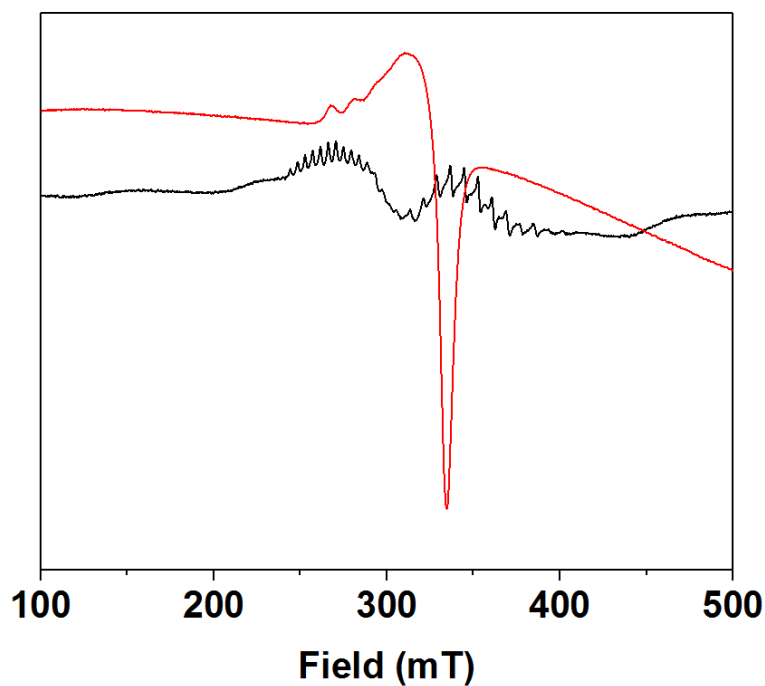


Figure A4.1: Perpendicular mode EPR spectra of **2** in 1:9 CH₃CN/THF (obtained from the reaction of 1.5 mM **1** with KO₂ in 1:9 CH₃CN/THF-black line) and Cu(NO₃)₂ standard (0.25 mM) in CH₃OH (red line) (2 K, 9.64 GHz, 0.2 mW microwave power).

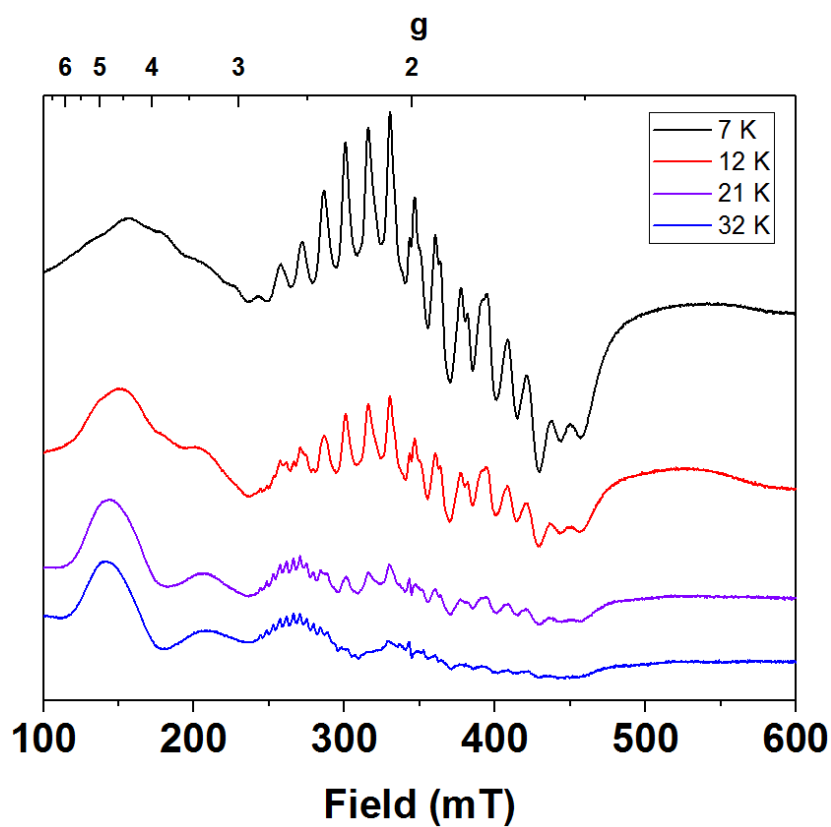


Figure A4.2: Perpendicular X-Band EPR spectrum of **2** (generated from 1.5 mM Mn₂BPMP + 1 eq. KO₂) measured at 7 K (black), 12 K (red), 21 K (purple), 32 K (blue) (9.65 GHz, 0.2 mW microwave power).

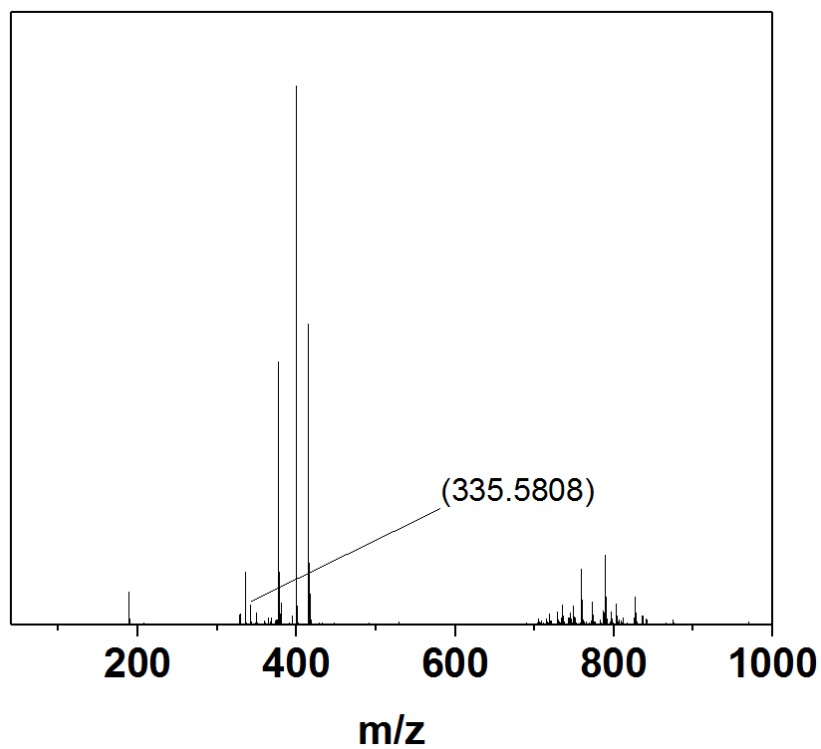


Figure A4.3: Mass spectrometry spectrum of the post-reaction mixture **2** + KO_2 + Heated -90 °C to +20 °C. Highlighted peak corresponds to **5**.

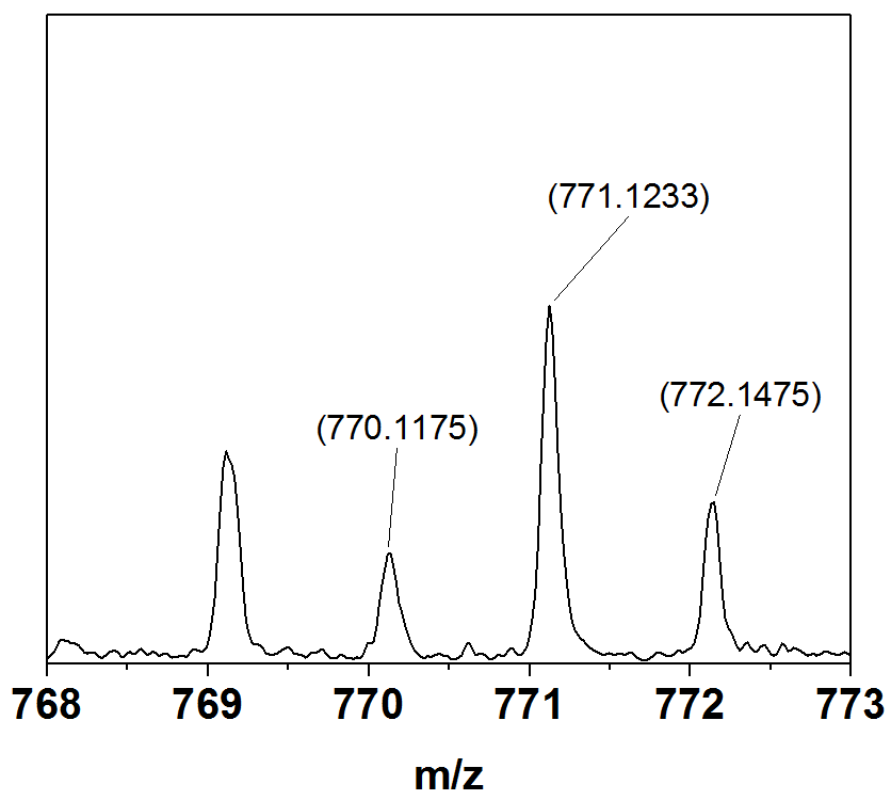


Figure A4.4: ESI-MS spectra of the post-reaction mixture of **4** expanded in the region where **2** would be expected. (**2** m/z = 770.29).

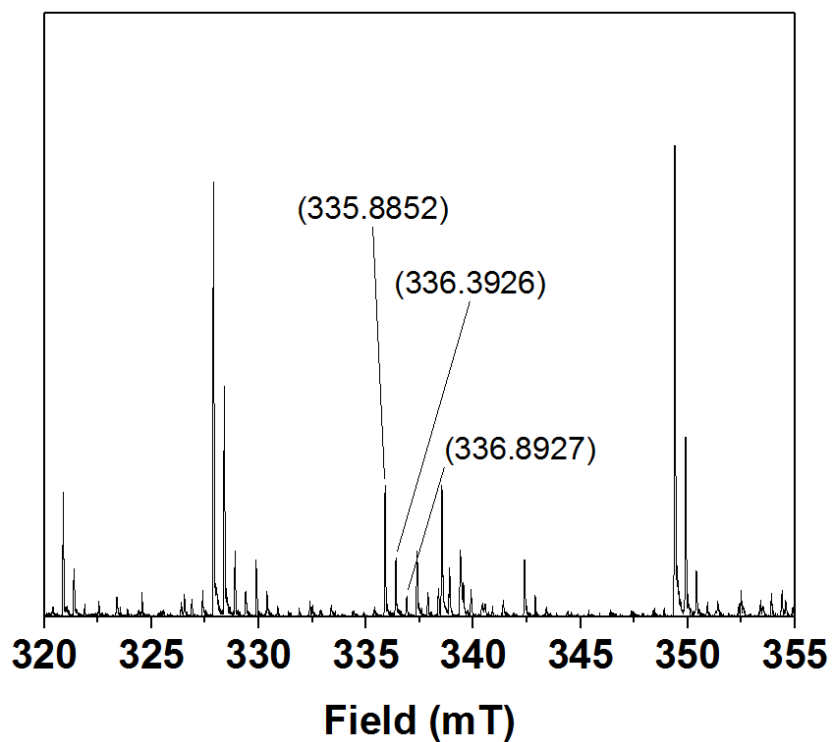


Figure A4.5: ESI-MS spectra of the post-reaction mixture of **2** expanded in the region where **5** would be expected. (**5** m/z = 335.5808)

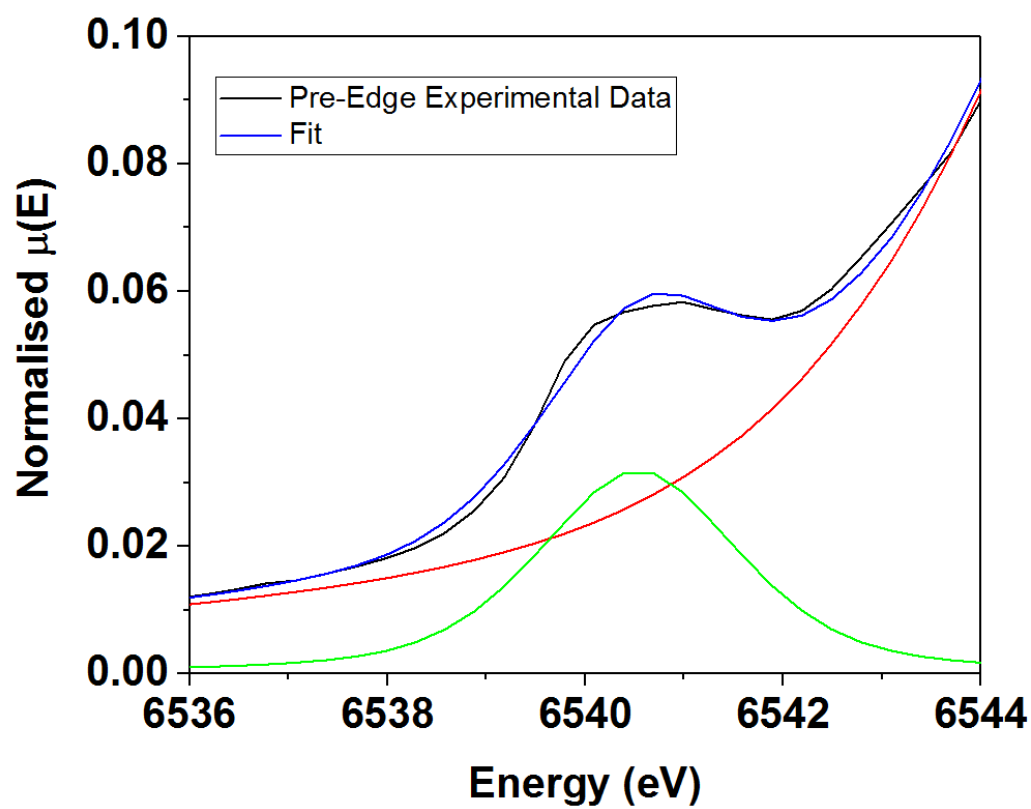


Figure A4.5: Rising edge and peak fitting of the pre-edge area of **5** with a 50:50 pseudo-Voigt function.

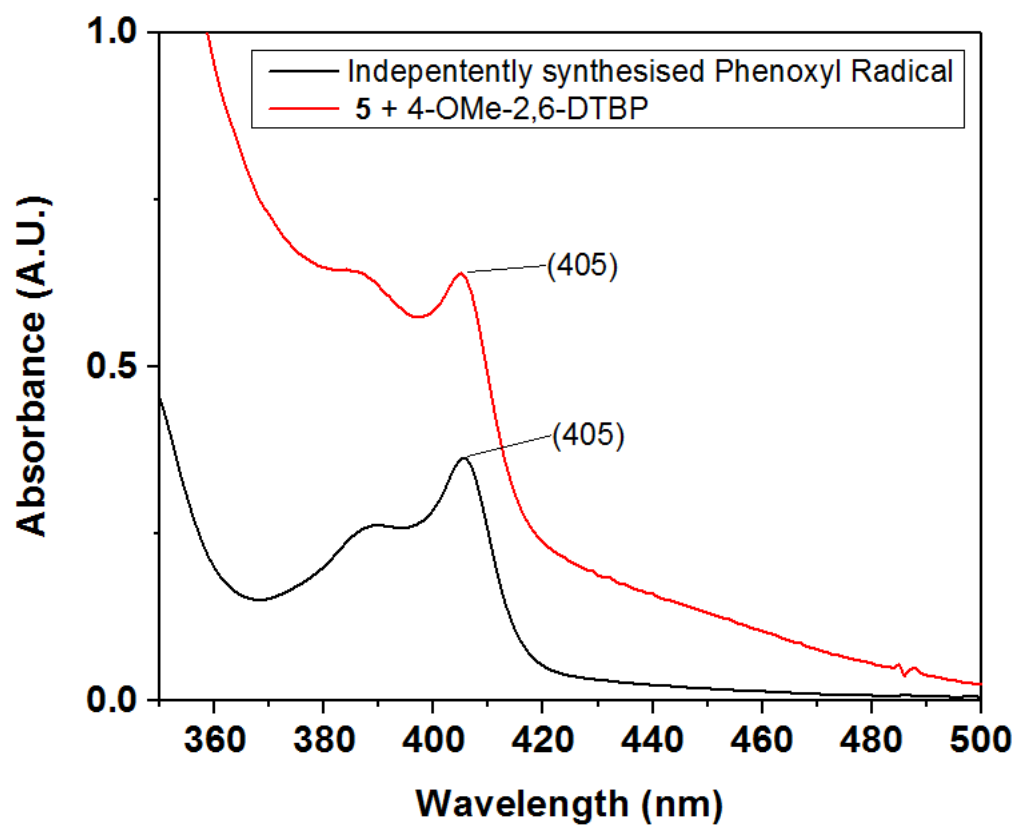


Figure A4.6: The electronic absorption spectra of the independently synthesised 4-methoxy-2,6-di-tert-butylphenoxyl radical (black). The electronic absorption spectra of the reaction of 5 with 4-OCH₃-2,6-DTBP (red).

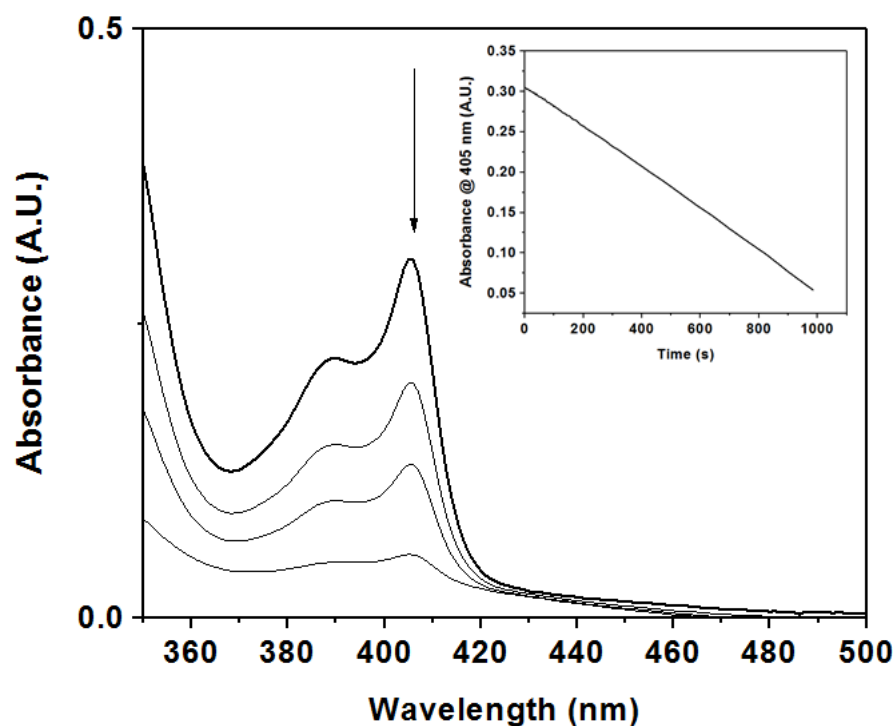


Figure A4.7: The decay of the electronic absorption features of 0.8 mM 4-methoxy-2,6-di-tert-butylphenoxyl radical in 1:9 MeCN/THF at 20 °C. Inset: Decay of the feature at λ_{nm} = 405 nm over time.

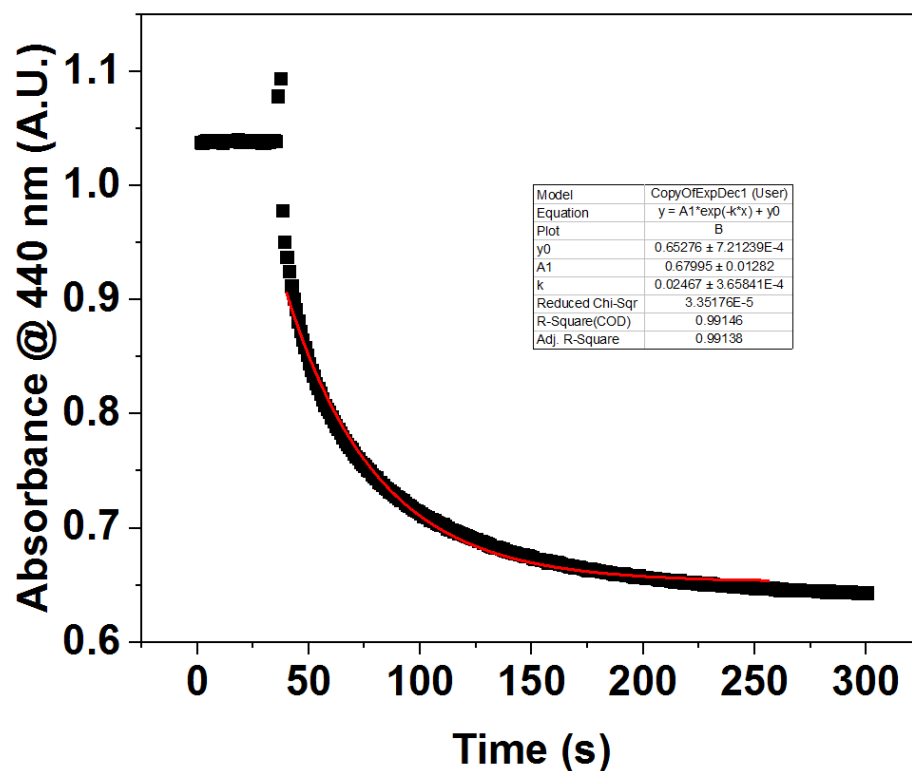


Figure A4.8: Plot of the absorbance at 440 nm during the reaction of **5** and 20 eq. 4-OCH₃-2,6-DTBP (black trace). Pseudo first-order fit of the decay (red trace)

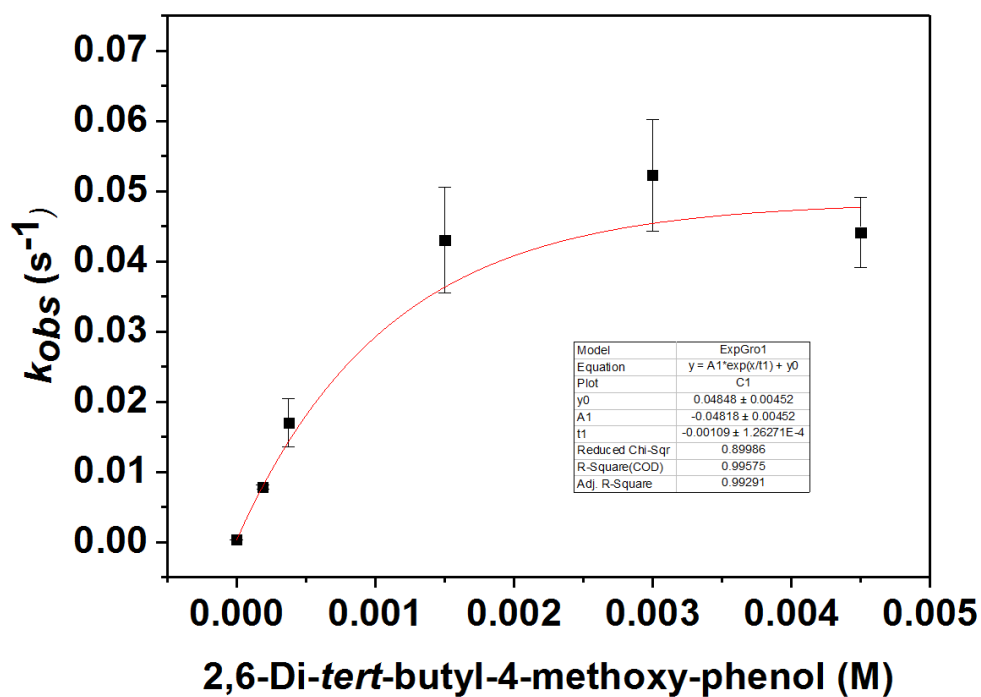


Figure A4.9: Plot of k_{obs} versus [4-OCH₃-2,6-DTBP] determined for the reaction of **5** and 4-OCH₃-2,6-DTBP.

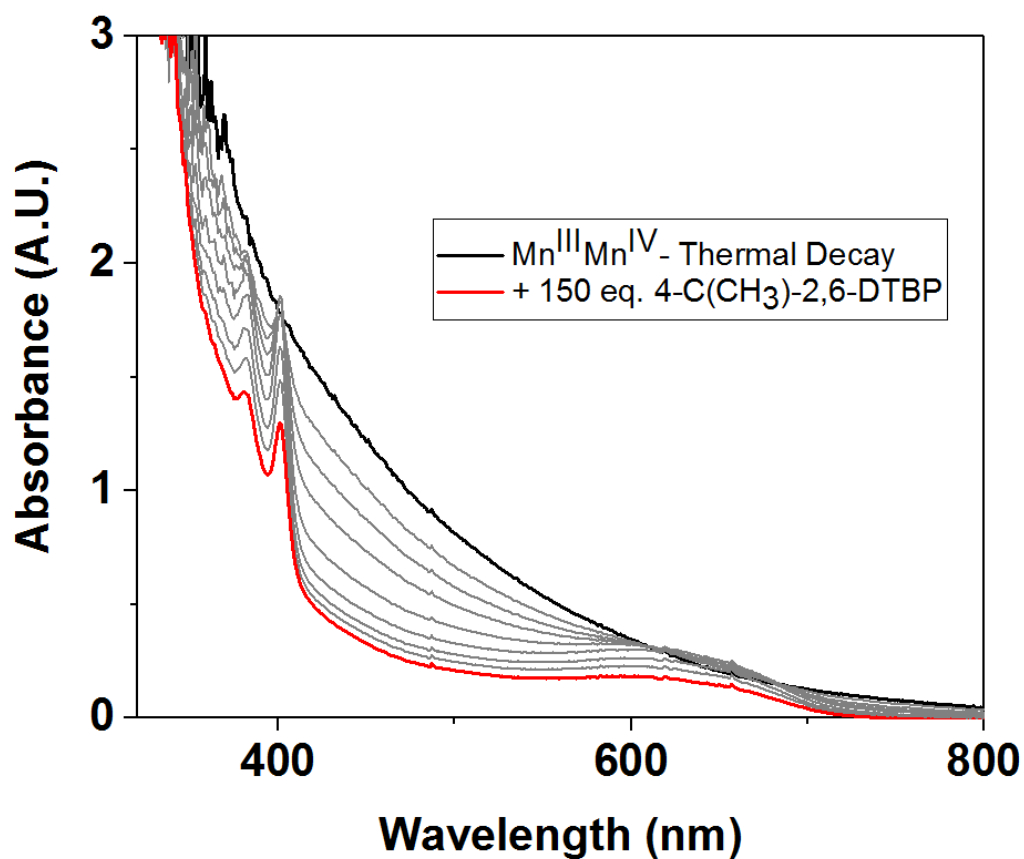


Figure A4.10: Electronic absorption features of the reaction of 5 and 150 eq. 4- $\text{C}(\text{CH}_3)_3$ -2,6-DTBP in 1:9 MeCN/THF measured at +20 °C.

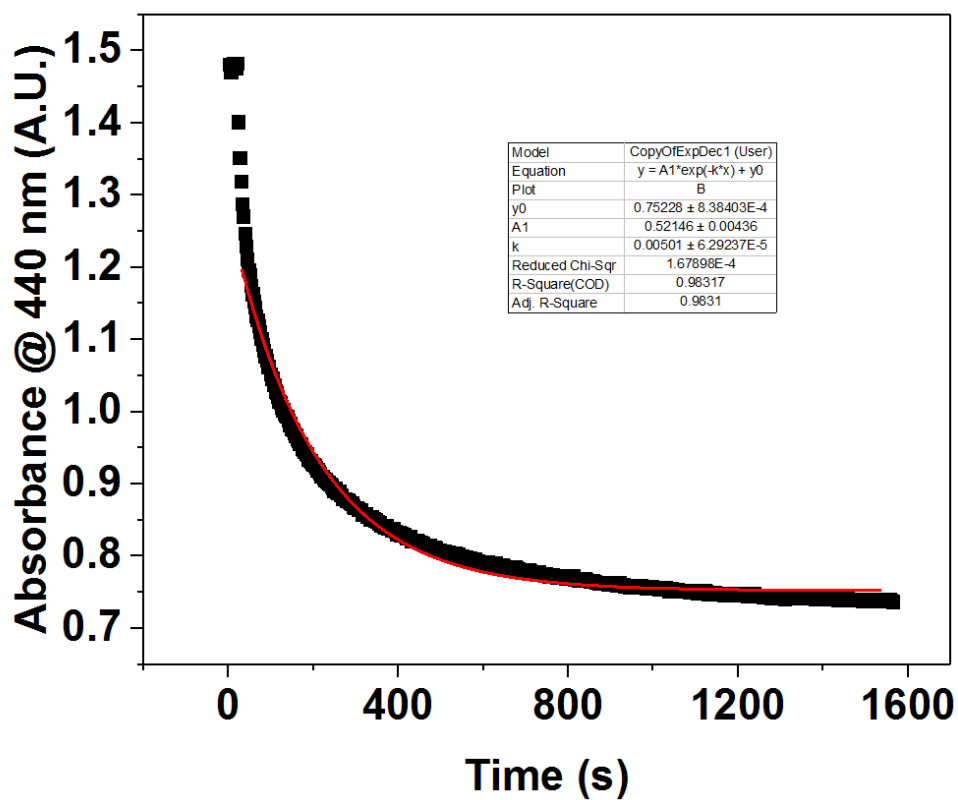


Figure A4.11: Plot of the absorbance at 440 nm during the reaction of 5 and 25 eq. 4- $\text{C}(\text{CH}_3)_3$ -2,6-DTBP (black trace). Pseudo first-order fit of the decay (red trace)

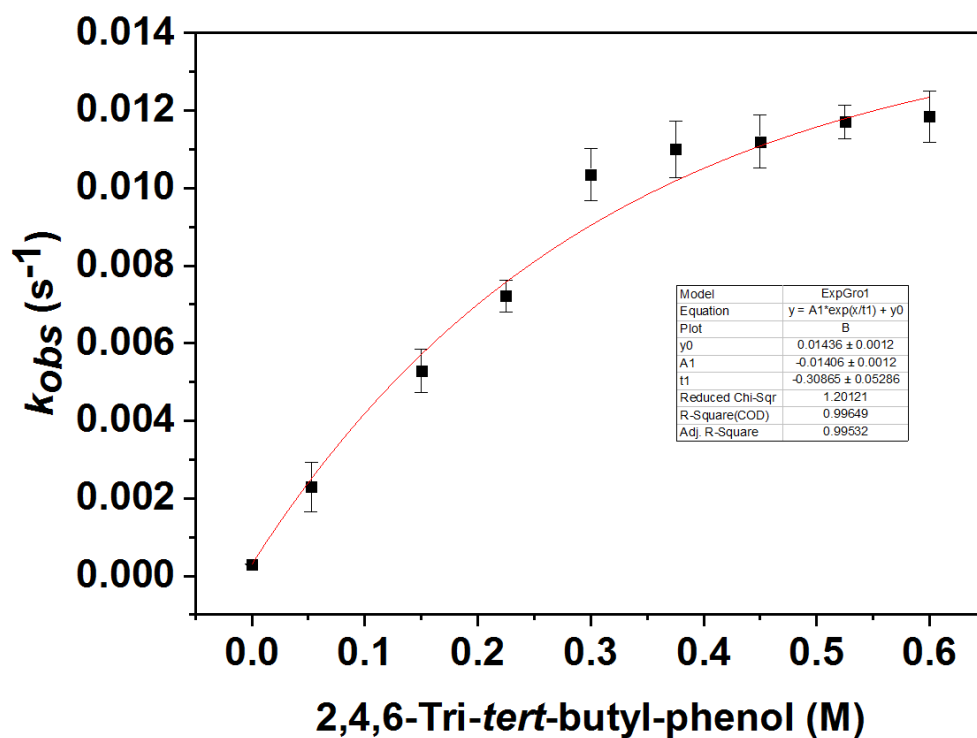


Figure A4.12: Plot of k_{obs} versus $[4-C(CH_3)_3-2,6-DTBP]$ determined for the reaction of **5** and 4- $(CH_3)_3-2,6-DTBP$.

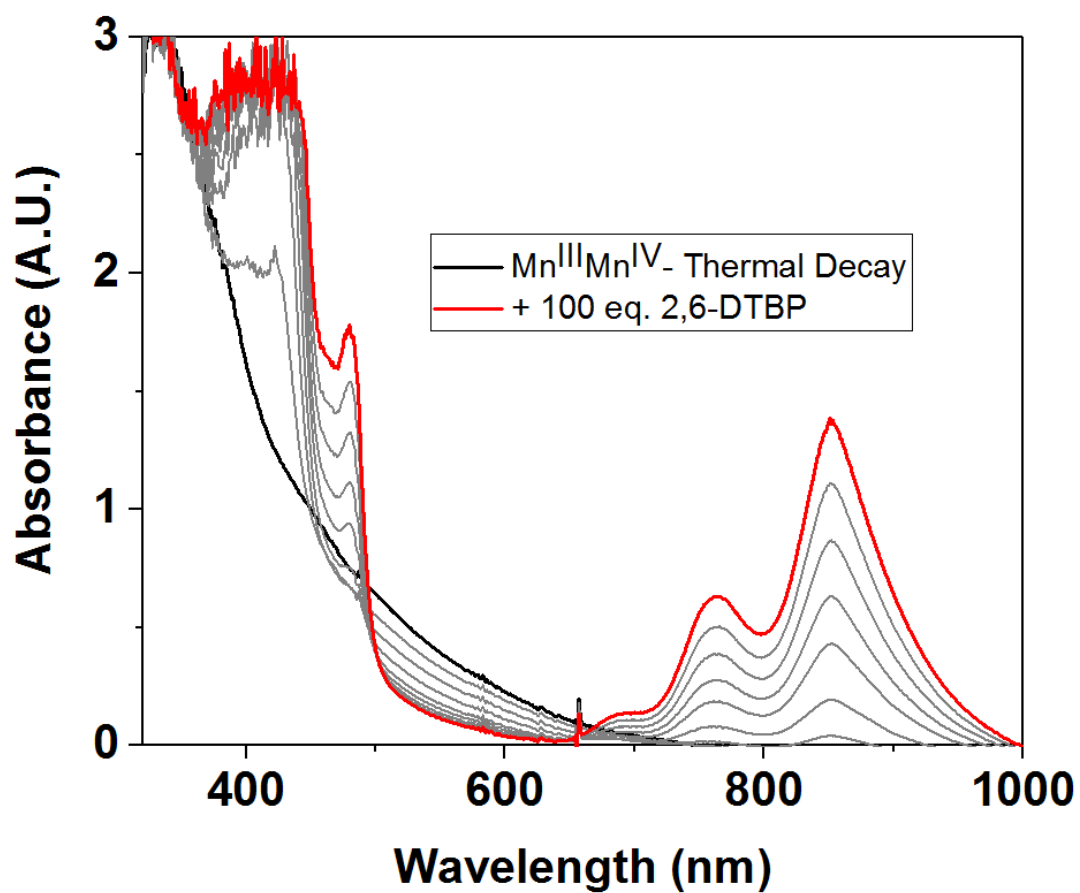


Figure A4.13: Electronic absorption features of the reaction of **5** and 100 eq. 2,6-DTBP in 1:9 MeCN/THF measured at +20 °C.

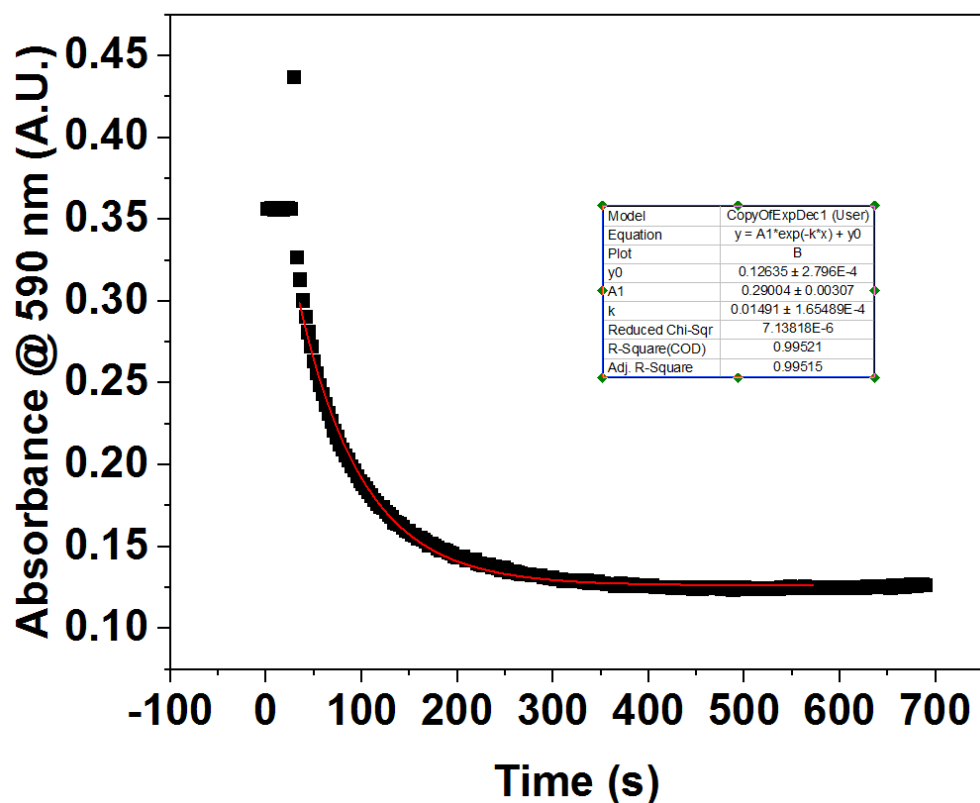


Figure A4.14: Plot of the absorbance at 590 nm during the reaction of **5** and 25 eq. 2,6-DTBP (black trace). Pseudo first-order fit of the decay (red trace).

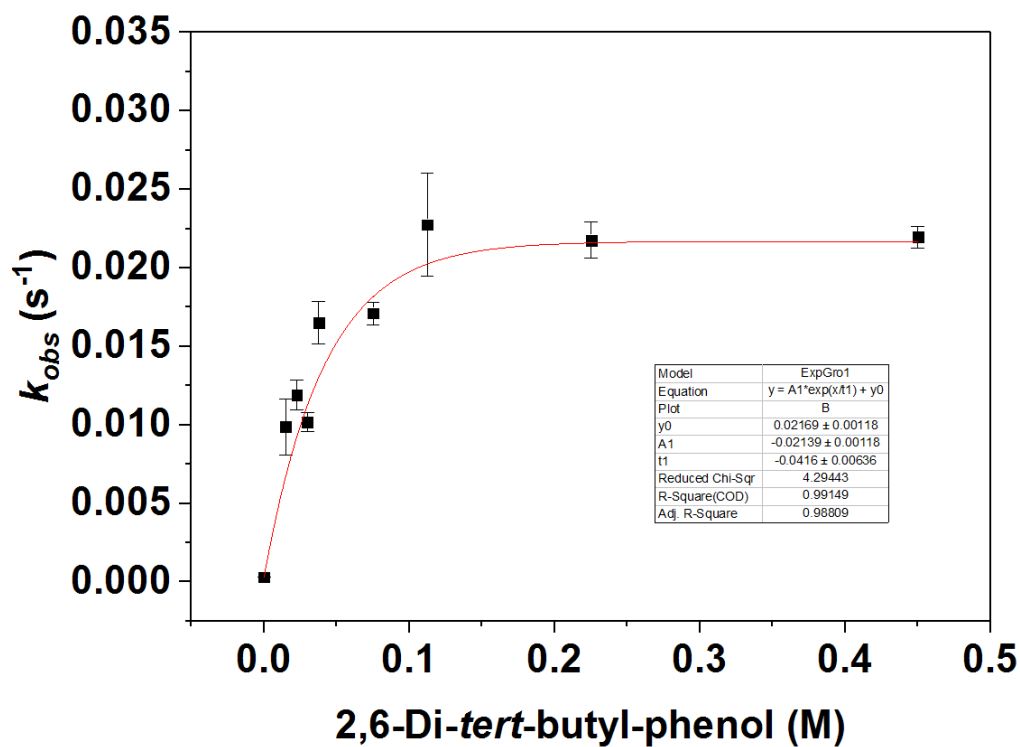


Figure A4.15: Plot of k_{obs} versus [2,6-DTBP] determined for the reaction of **5** and 2,6-DTBP.

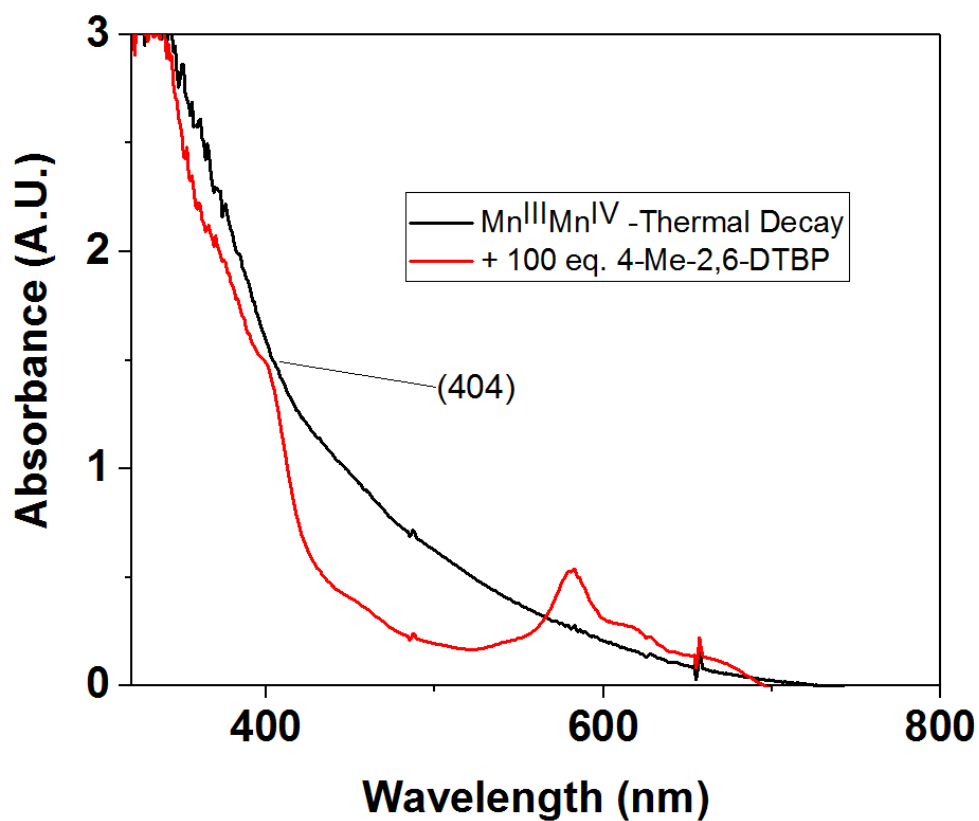


Figure A4.16: Electronic absorption features of the reaction between **5** and 100 eq. 4-Me-2,6-DTBP in 1:9 MeCN/THF measured at +20 °C.

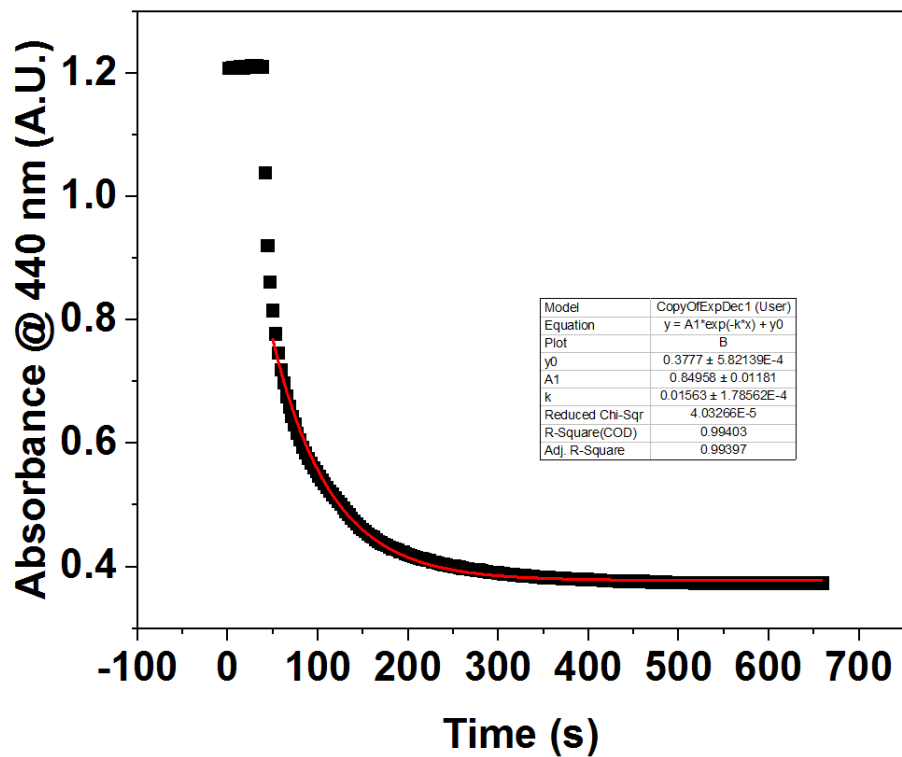


Figure A4.17: Plot of the absorbance at 440 nm during the reaction of **5** and 150 eq. 4-CH₃-2,6-DTBP (black trace). Pseudo first-order fit of the decay (red trace)

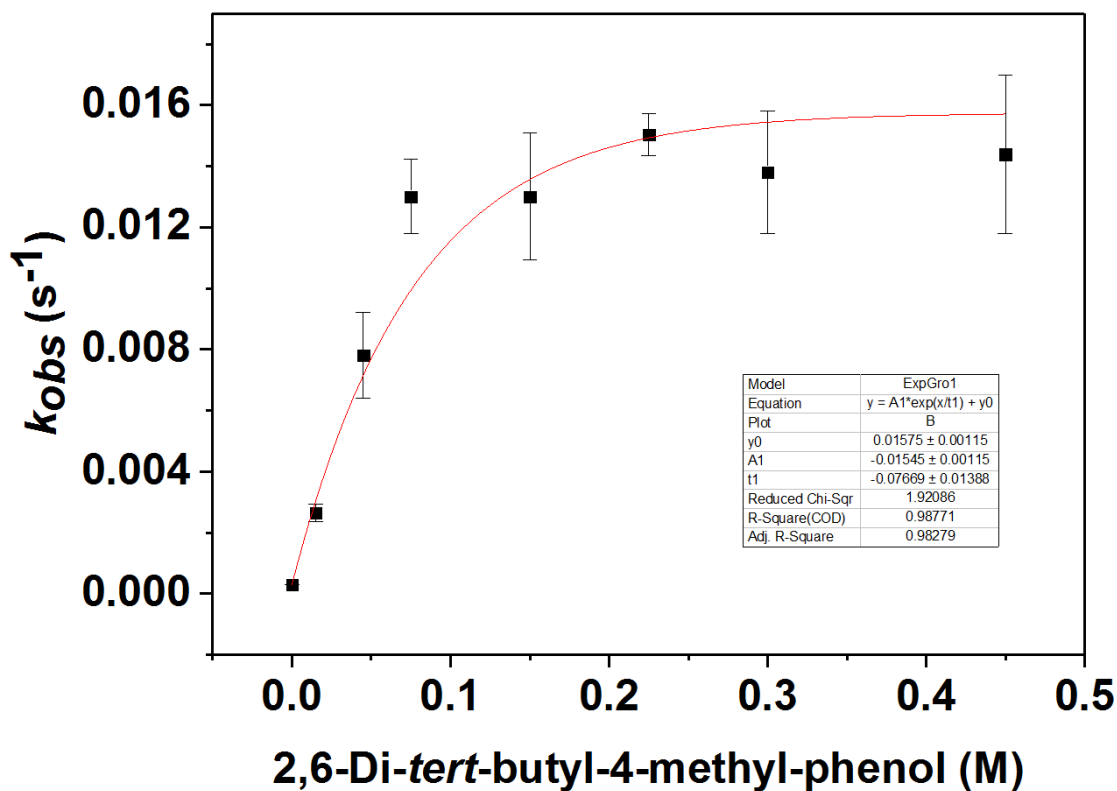


Figure A4.18: Plot of k_{obs} versus [4-Me-2,6-DTBP] determined for the reaction of 5 and 4-Me-2,6-DTBP.

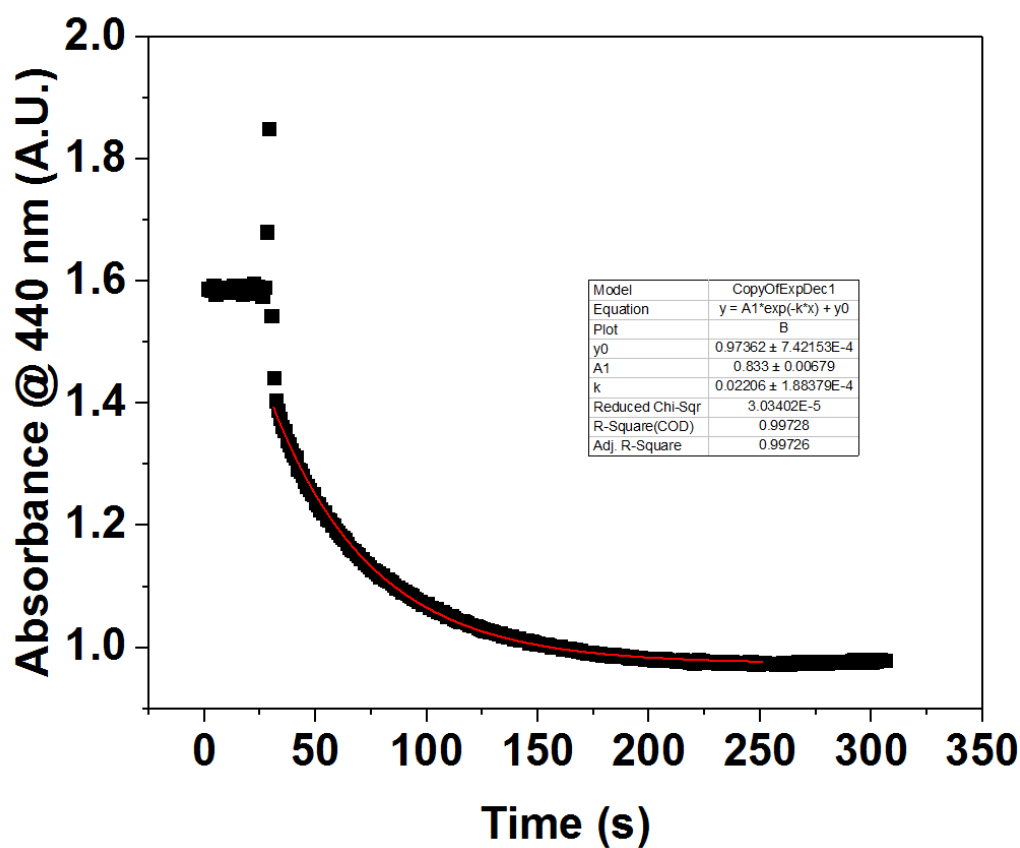


Figure A4.19: Plot of the absorbance at 440 nm during the reaction of 5 and 75 eq. 4-C₂H₅-2,6-DTBP (black trace). Pseudo first-order fit of the decay (red trace)

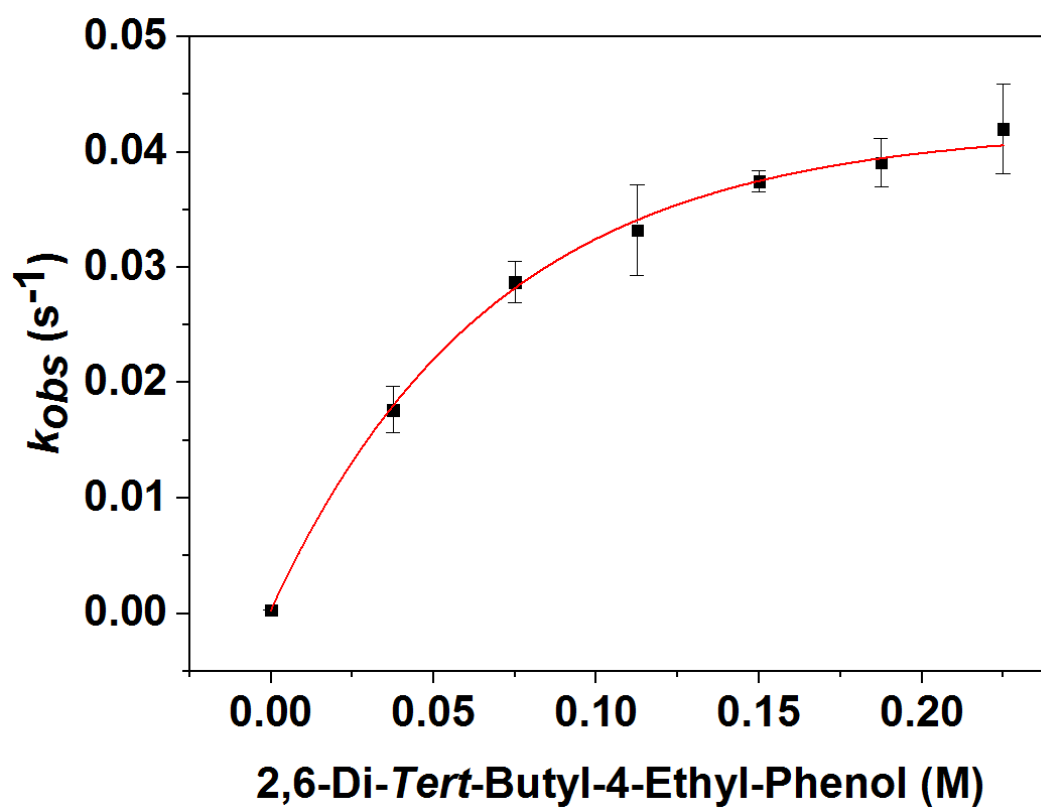


Figure A4.20: Plot of k_{obs} versus $[4\text{-C}_2\text{H}_5\text{-2,6-DTBP}]$ determined for the reaction of **5** and 4- $\text{C}_2\text{H}_5\text{-2,6-DTBP}$.

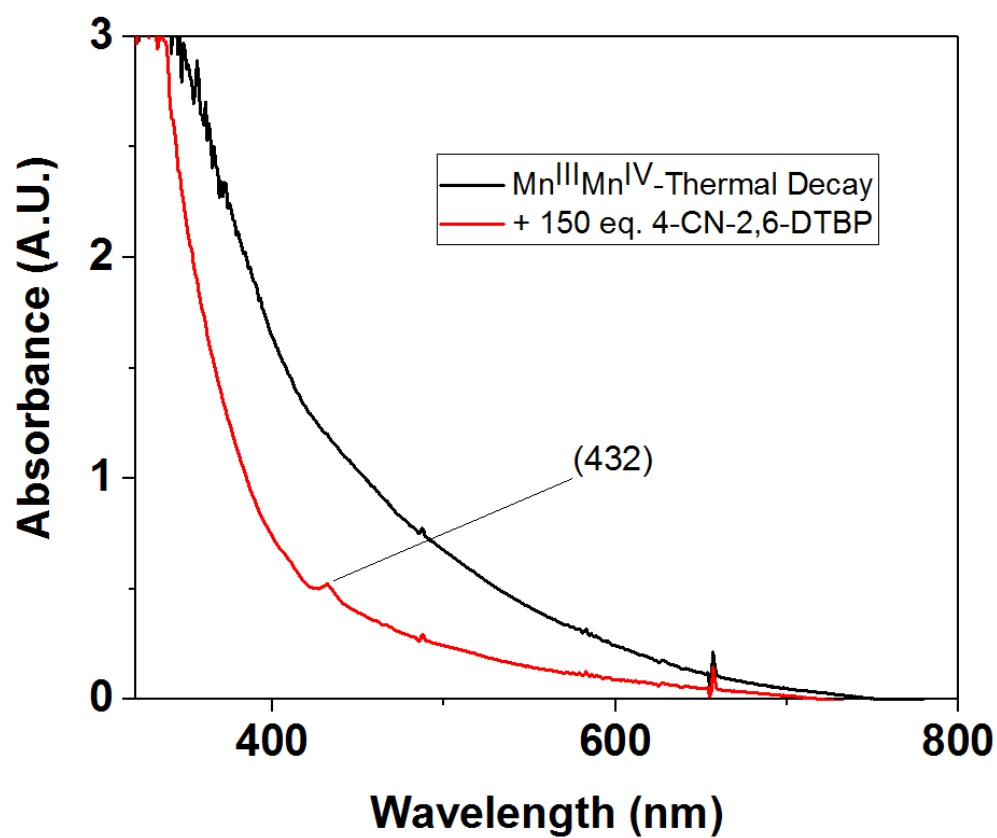


Figure A4.21: Electronic absorption features of the reaction between **5** and 150 eq. 4-CN-2,6-DTBP in 1:9 MeCN/THF measured at +20 °C.

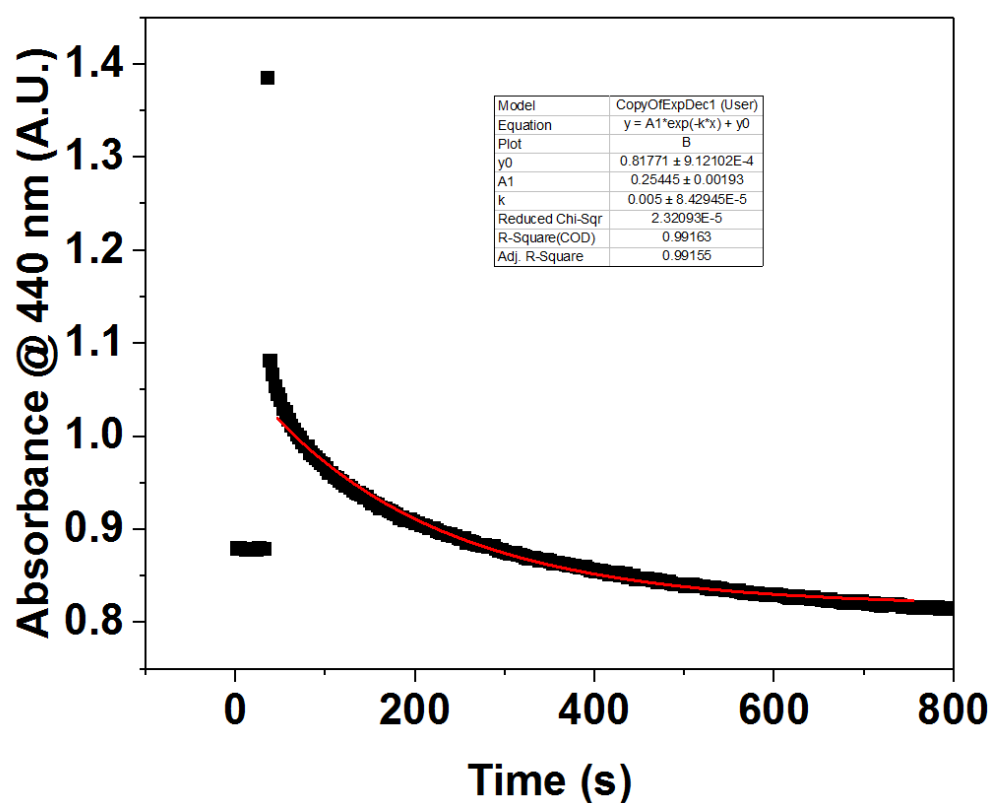


Figure A4.22: Plot of the absorbance at 440 nm during the reaction of **5** and 350 eq. 4-CN-2,6-DTBP (black trace). Pseudo first-order fit of the decay (red trace)

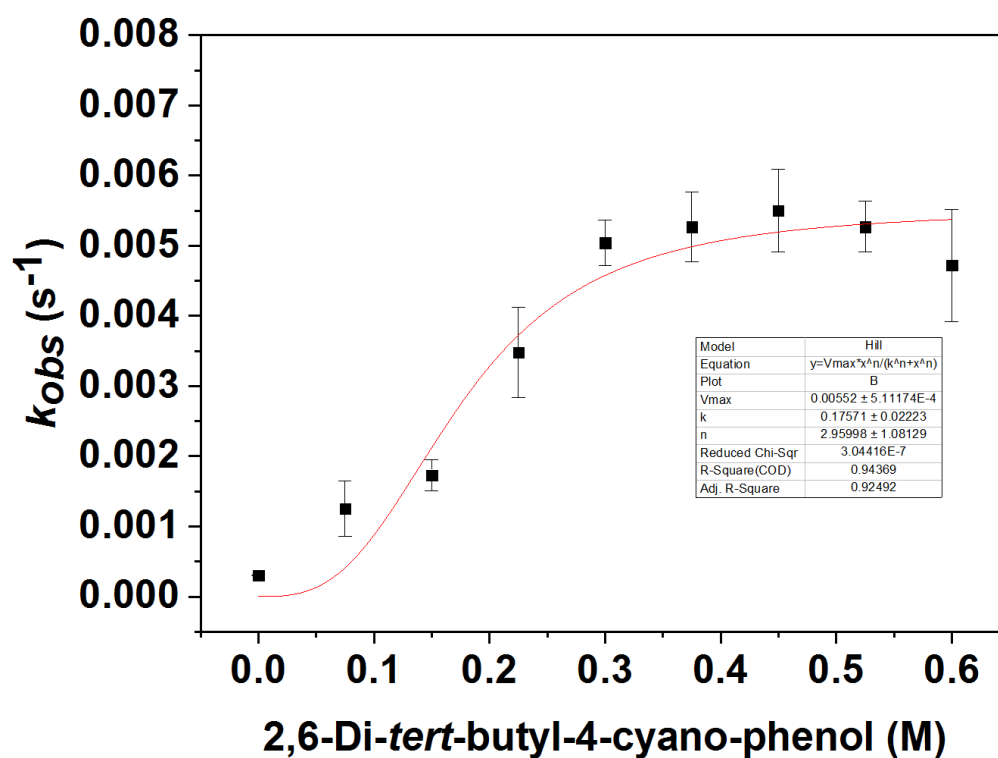


Figure A4.23: Plot of k_{obs} versus [4-CN-2,6-DTBP] determined for the reaction of **5** and 4-CN-2,6-DTBP.

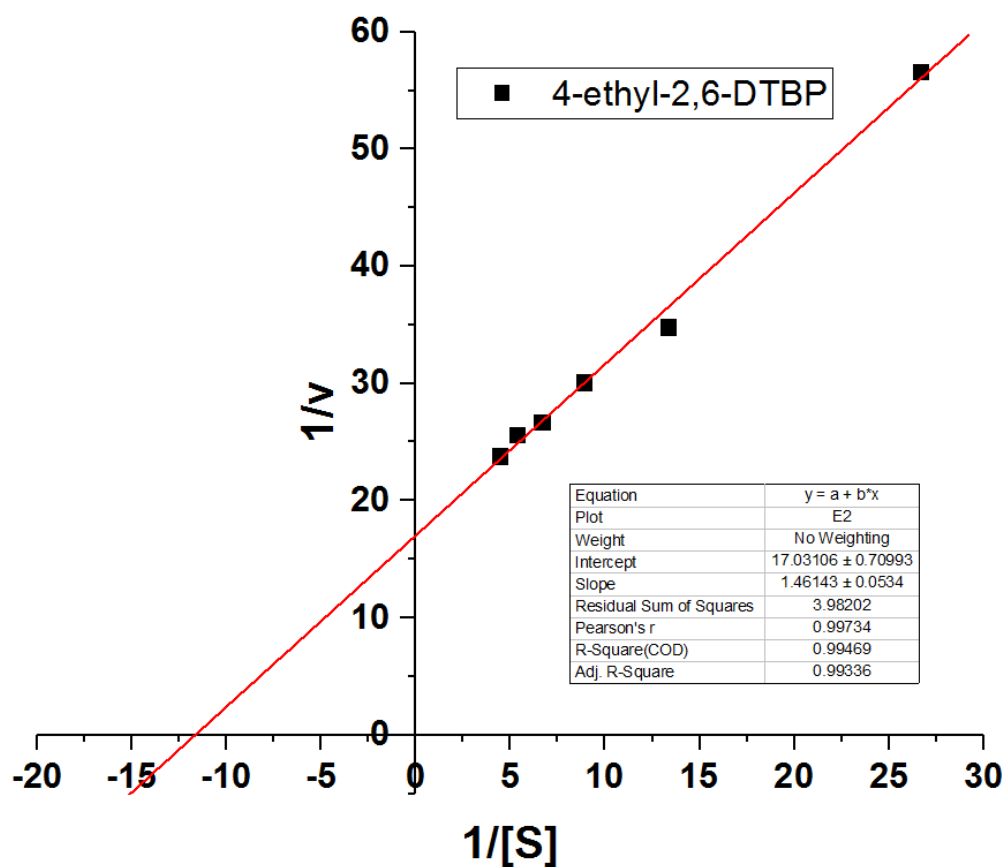


Figure A4.24: Plot of $1/k_{obs}$ versus $1/[4\text{-Et-2,6-DTBP}]$ determined for the reaction between **5** and 4-Et-2,6-DTBP.

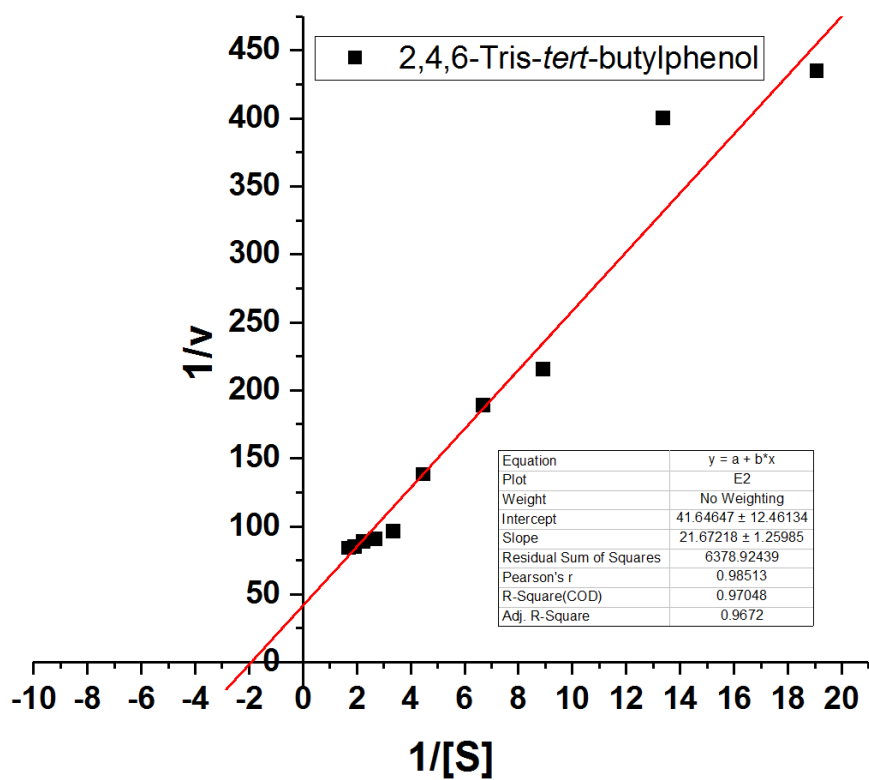


Figure A4.25: Plot of $1/k_{obs}$ versus $1/[4\text{-C(CH}_3)_3\text{-2,6-DTBP}]$ determined for the reaction between **5** and 4-C(CH₃)₃-2,6-DTBP.

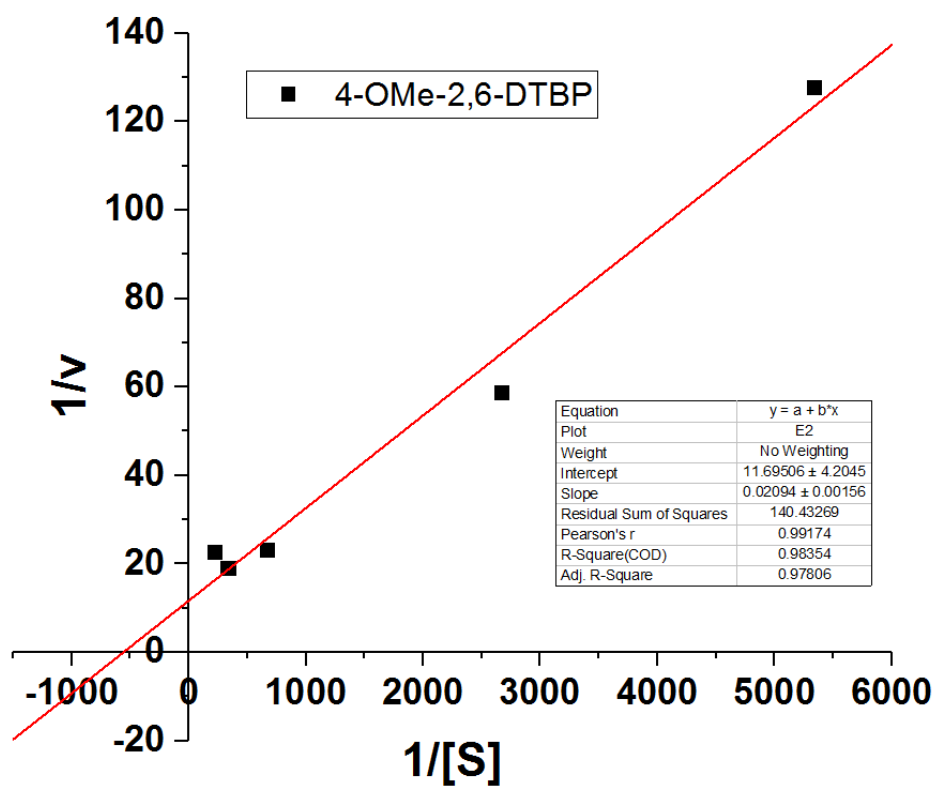


Figure A4.26: Plot of $1/k_{obs}$ versus $1/[4\text{-OCH}_3\text{-2,6-DTBP}]$ determined for the reaction between **5** and 4-OCH₃-2,6-DTBP.

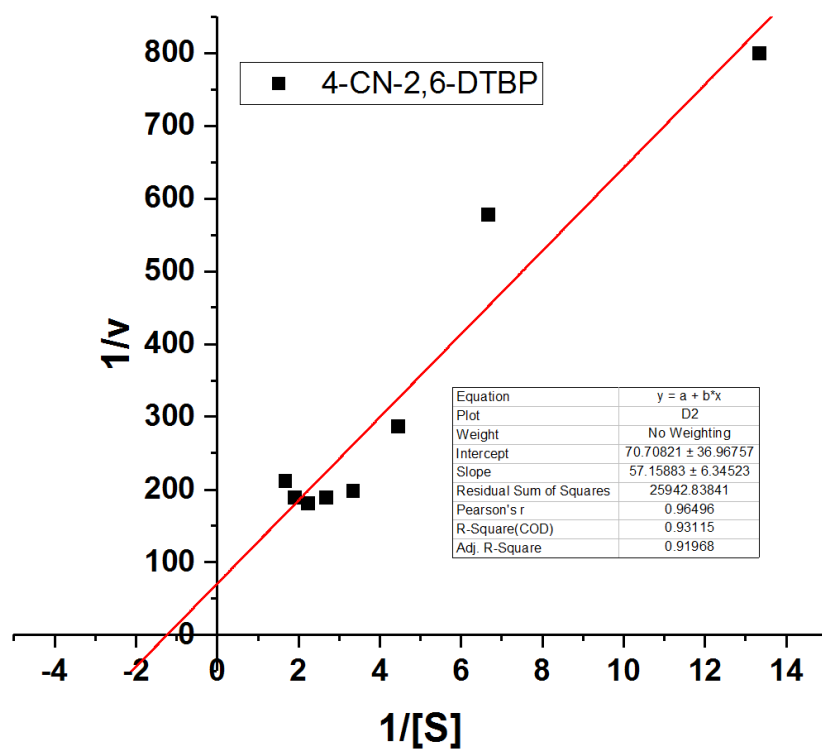


Figure A4.27: Plot of $1/k_{obs}$ versus $1/[4\text{-CN-2,6-DTBP}]$ determined for the reaction between **5** and 4-CN-2,6-DTBP.

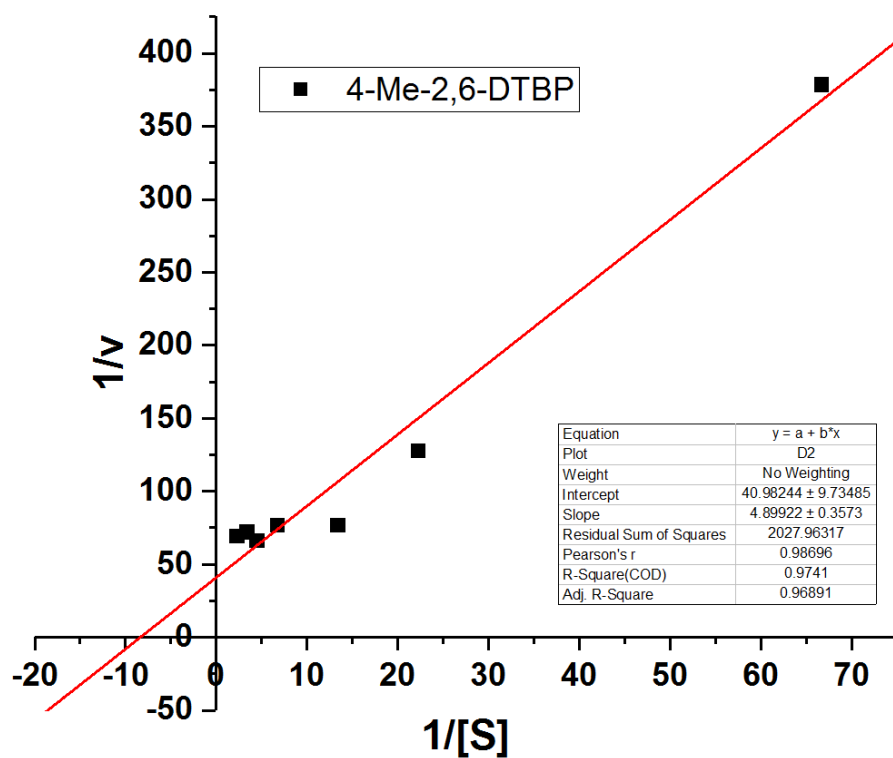


Figure A4.28: Plot of $1/k_{obs}$ versus $1/[4\text{-Me-2,6-DTBP}]$ determined for the reaction between **5** and 4-Me-2,6-DTBP.

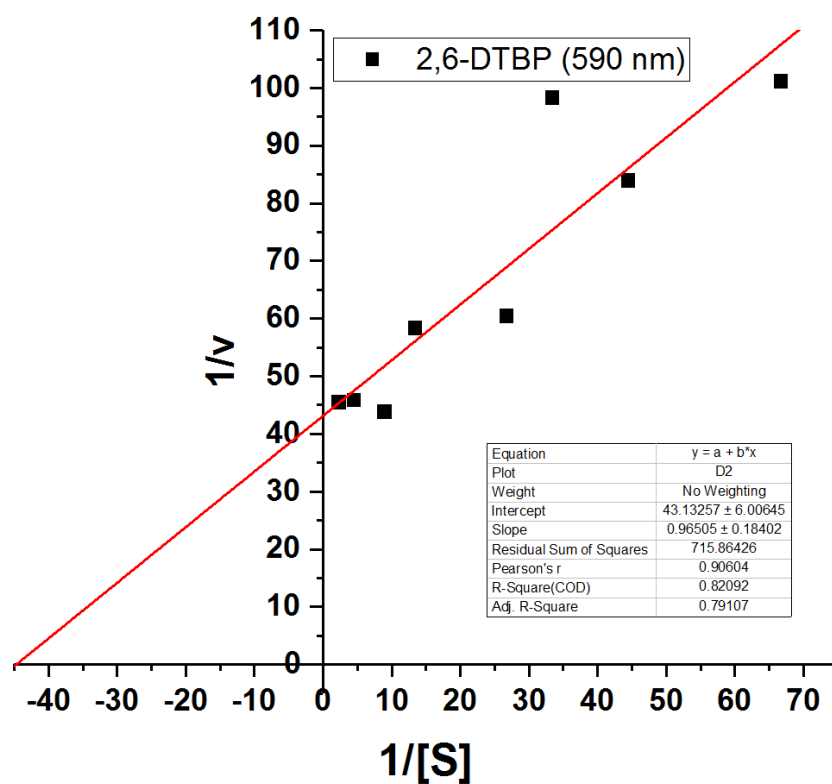


Figure A4.29: Plot of $1/k_{obs}$ versus $1/[2,6\text{-DTBP}]$ determined for the reaction between **5** and 2,6-DTBP.

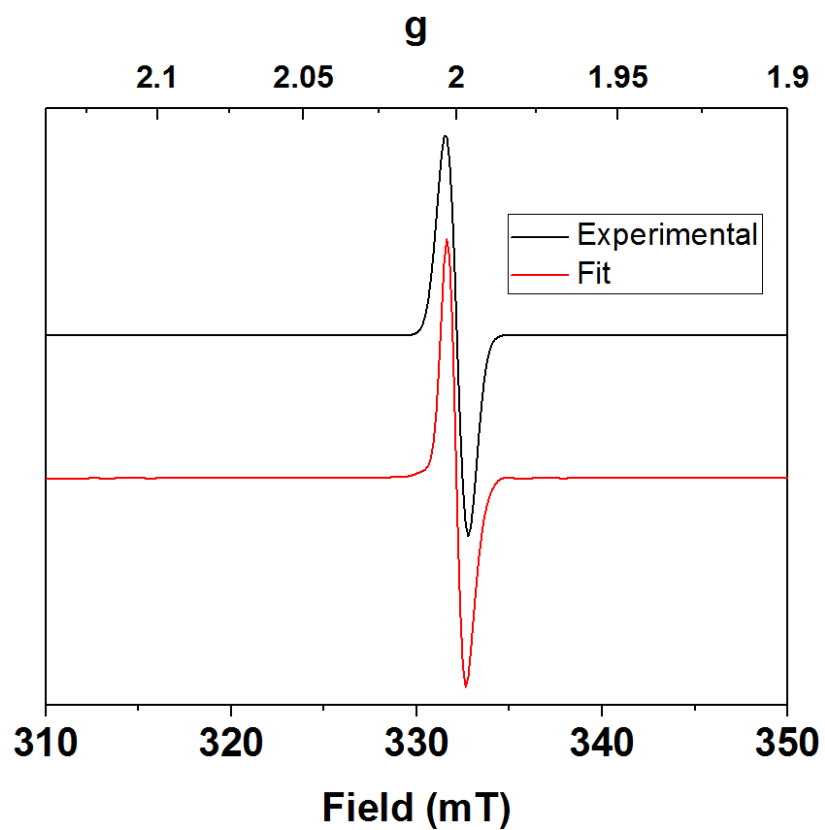


Figure A4.30: EPR spectra of the post-reaction mixture of 4 and 4-OCH₃-2,6-DTBP

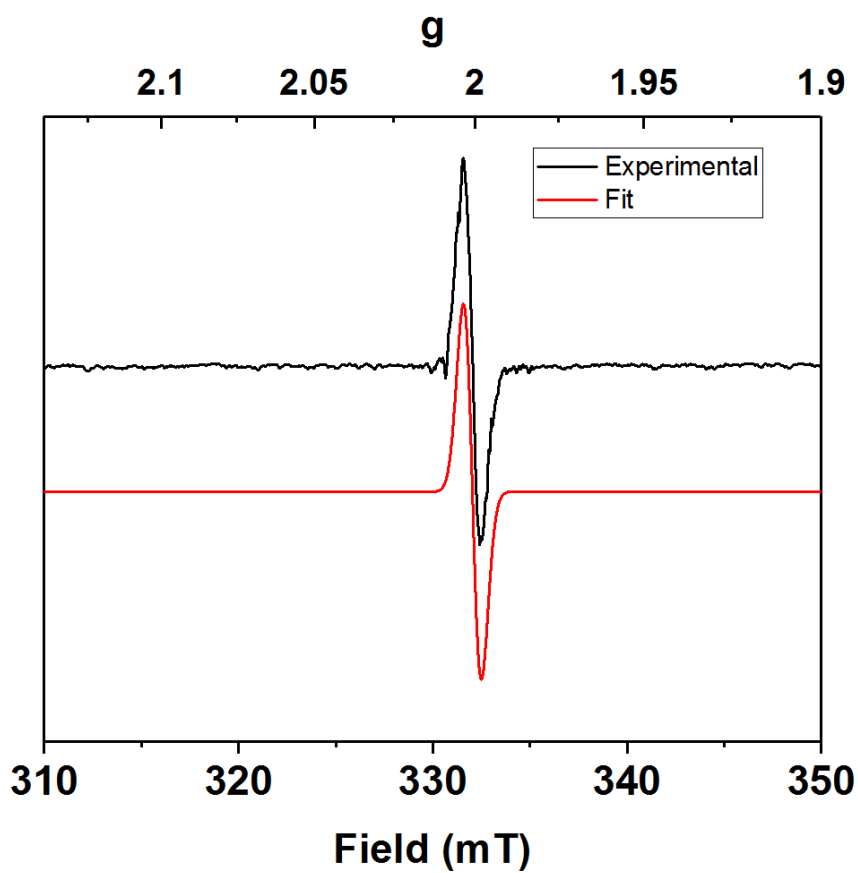


Figure A4.31: EPR spectra of the post-reaction mixture of 5 and 4-C(CH₃)₃-2,6-DTBP

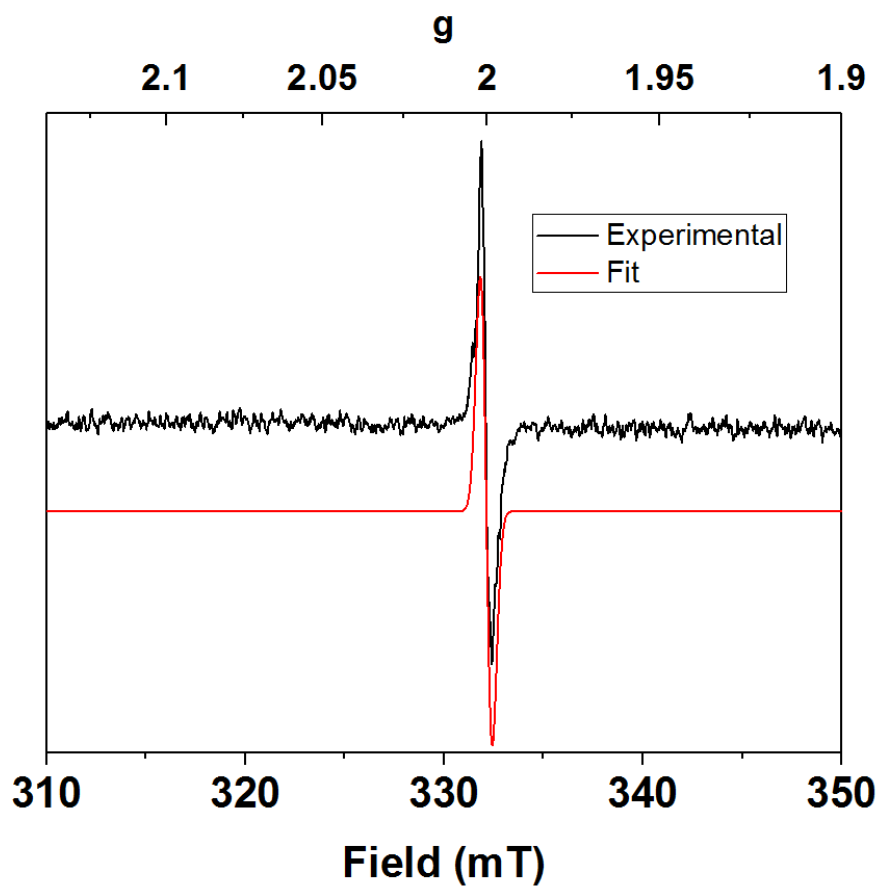


Figure A4.32: EPR spectra of the post-reaction mixture of 5 and 2,6-DTBP

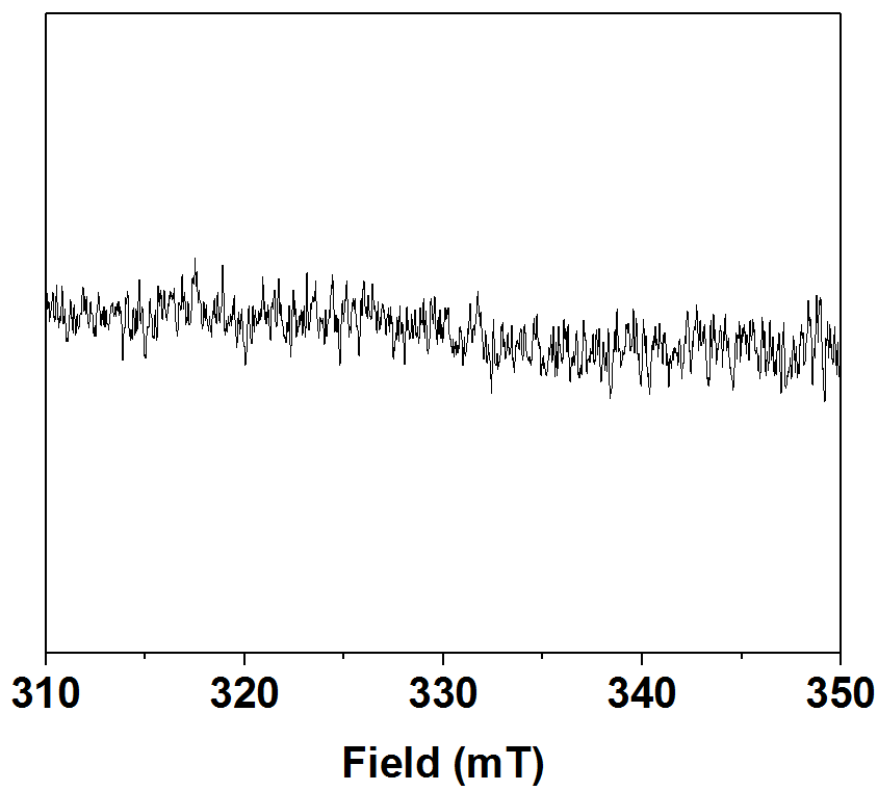


Figure A4.33: EPR spectra of the post-reaction mixture of 5 and 4-Me-2,6-DTBP

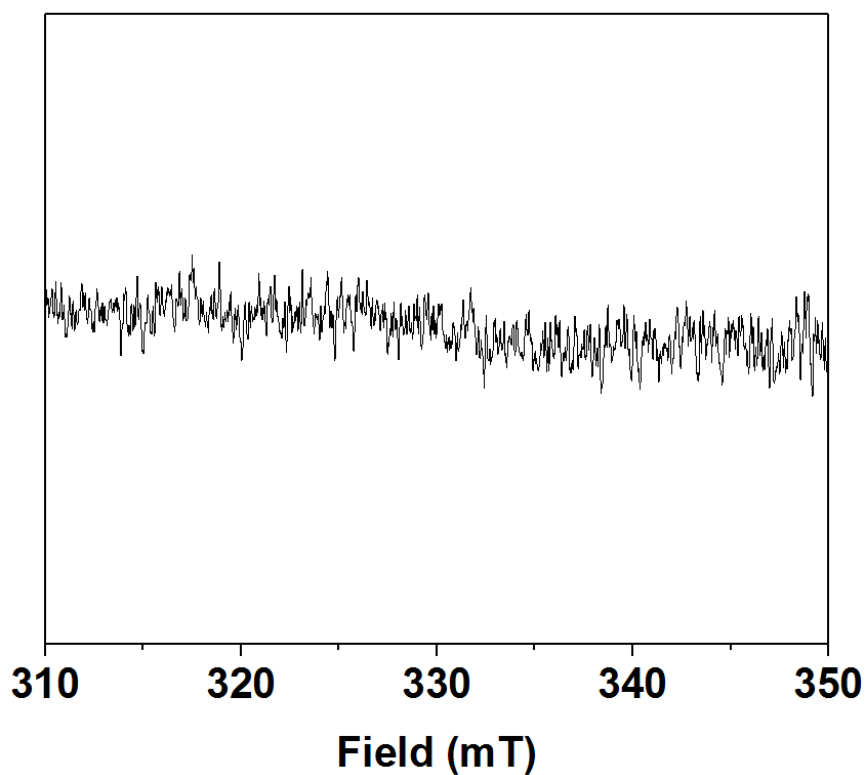


Figure A4.34: EPR spectra of the post-reaction mixture of **5** and 4-CN-2,6-DTBP

4-X-2,6-DTBP X =	EPR Radical Yield ($\pm 15\%$)
OCH ₃	70
C(CH ₃) ₃	20
H	10
CH ₃	-
CN	-

Figure A4.35: Yields of the radical species generated after reaction with **5** as determined by EPR spectroscopy.

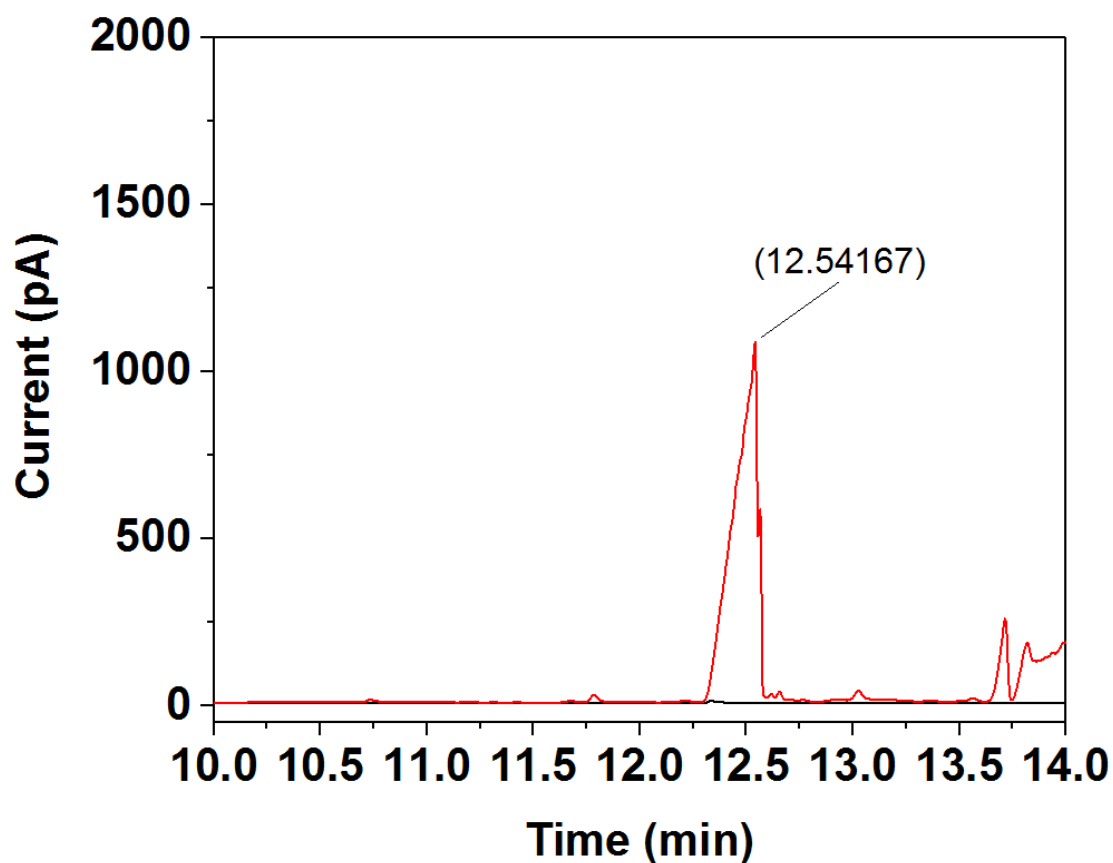


Figure A4.36: GC spectra of the post-reaction mixture of 5 + 4-OCH₃-2,6-DTBP (red) and a control reaction of 1:9 MeCN/THF + 4-OCH₃-2,6-DTBP (black trace). Annotated peak corresponded to 2,6-DTBQ product.

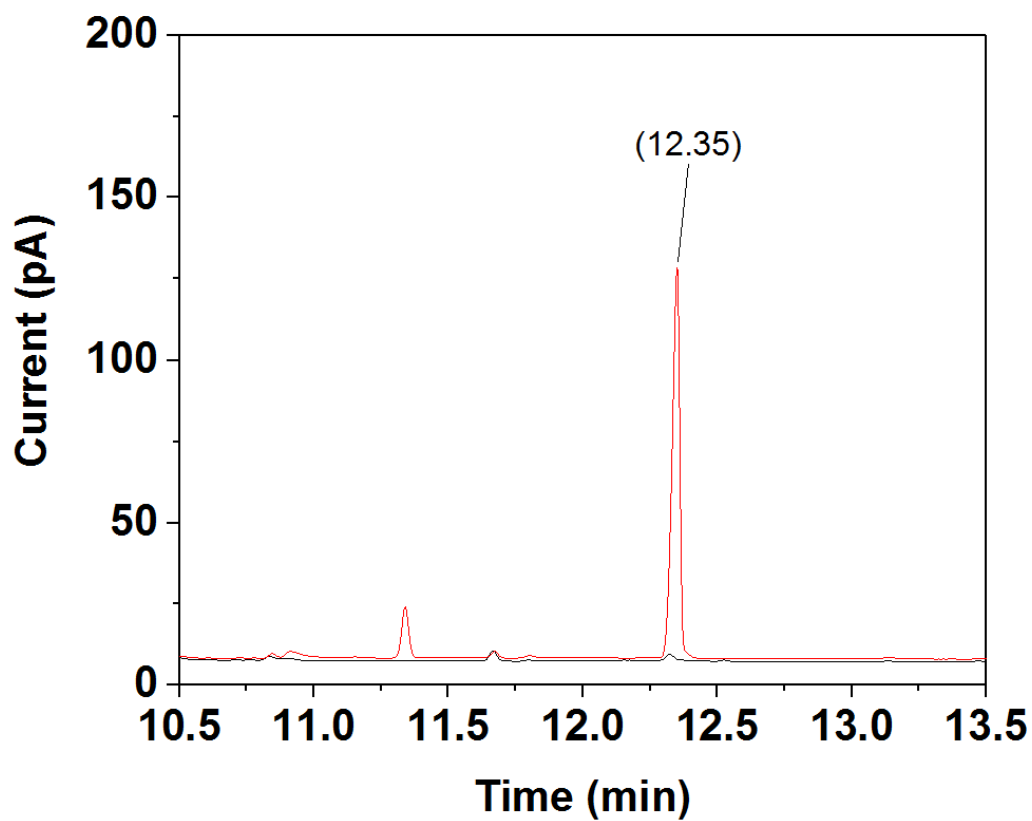


Figure A4.37: GC spectra of the post-reaction mixture of 5 + 4-CN-2,6-DTBP (red) and a control reaction of 1:9 MeCN/THF + 4-CN-2,6-DTBP (black trace). Annotated peak corresponded to 2,6-DTBQ product.

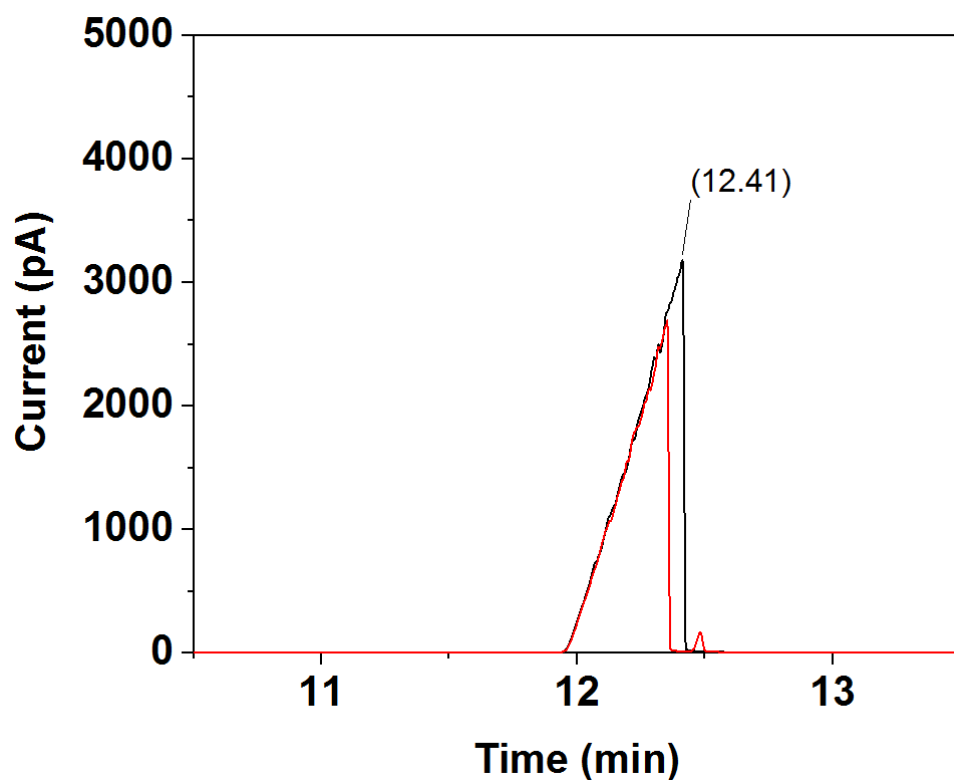


Figure A4.38: GC spectra of the post-reaction mixture of 5 + 2,6-DTBP (red) and a control reaction of 1:9 MeCN/THF + 2,6-DTBP (black trace). Annotated peak corresponded to 2,6-DTBQ product.

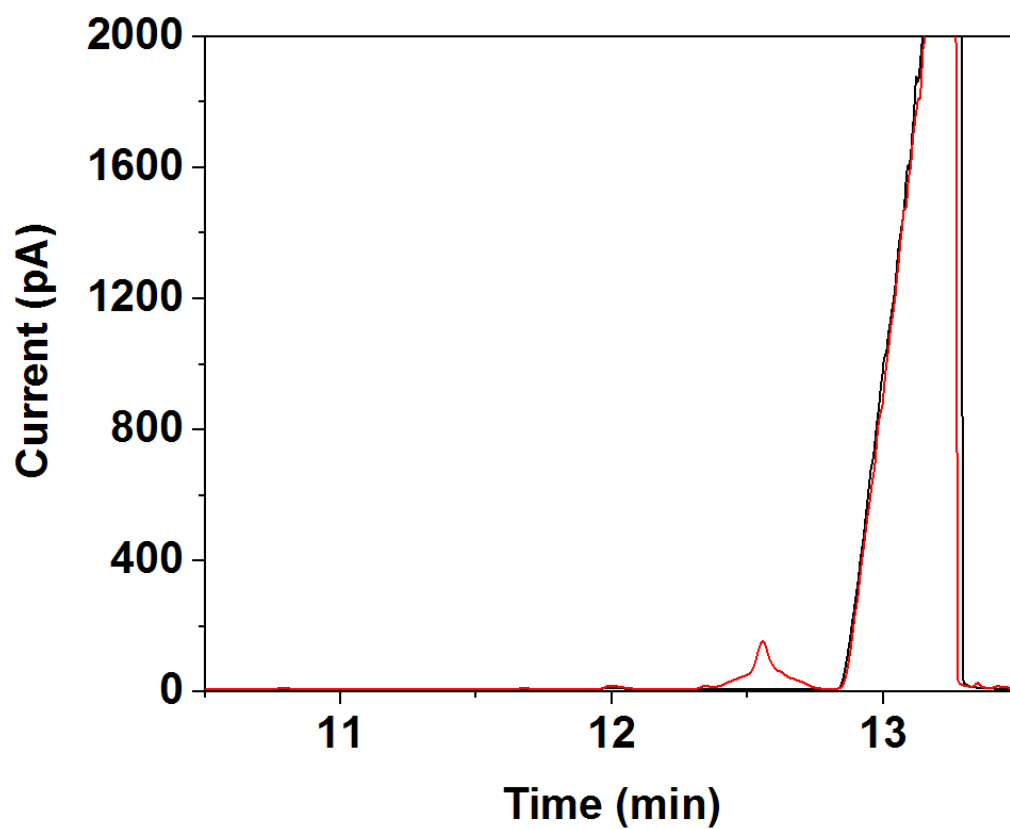


Figure A4.39: GC spectra of the post-reaction mixture of 5 + 4-CH₃-2,6-DTBP (red) and a control reaction of 1:9 MeCN/THF + 4-CH₃-2,6-DTBP (black trace).

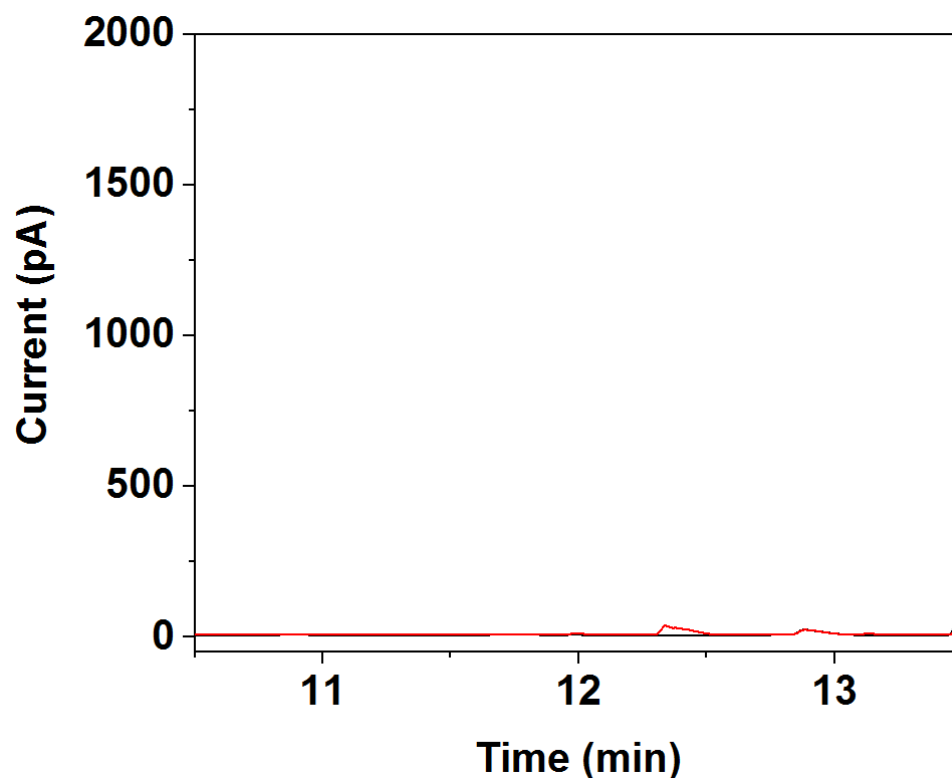


Figure A4.40: GC spectra of the post-reaction mixture of **5** + 4- C_2H_5 -2,6-DTBP (red) and a control reaction of 1:9 MeCN/THF + 4- C_2H_5 -2,6-DTBP (black trace).

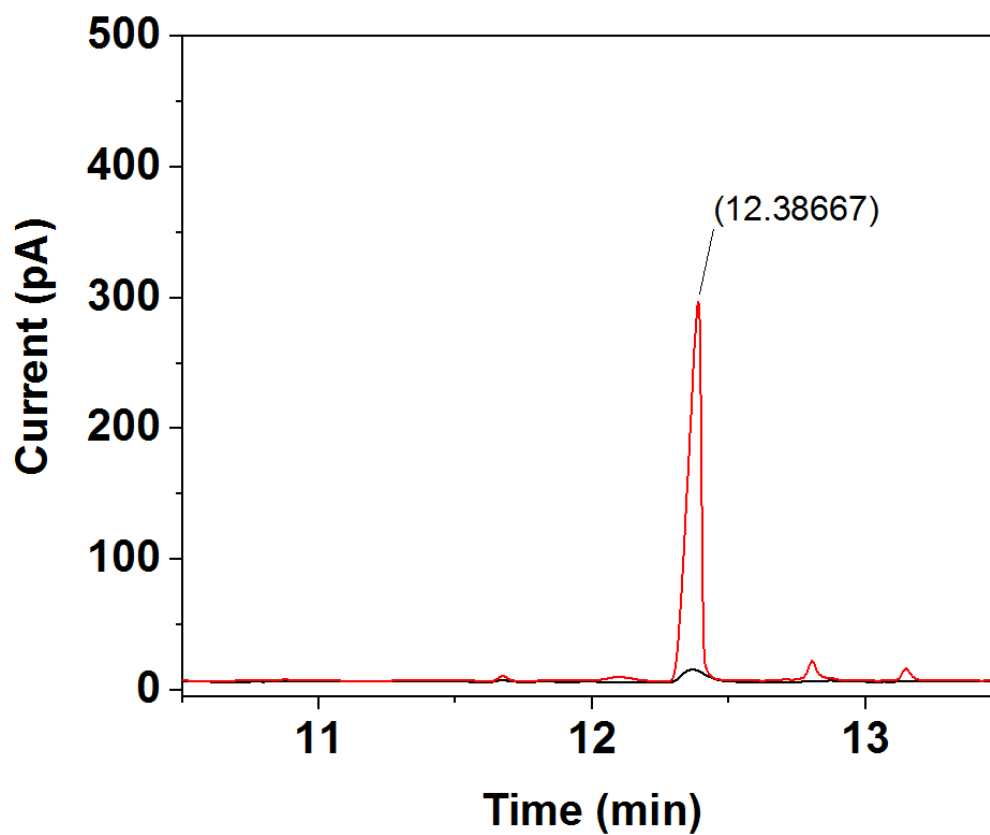


Figure A4.41: GC spectra of the post-reaction mixture of **5** + 4- $\text{C}(\text{CH}_3)_3$ - 2,6-DTBP (red) and a control reaction of 1:9 MeCN/THF + 4- $\text{C}(\text{CH}_3)_3$ -2,6-DTBP (black trace). Annotated peak corresponded to 2,6-DTBQ product.

4-X-2,6-DTBP X =	GC 2,6-DTBQ Yield ($\pm$ 60%)
OCH ₃	1330
C(CH ₃) ₃	185
H	-
CH ₃	-
CN	50
C ₂ H ₅	40

Figure A4.42: Yields of the 2,6-DTBQ generated after reaction with **5** as determined by GC spectroscopy.

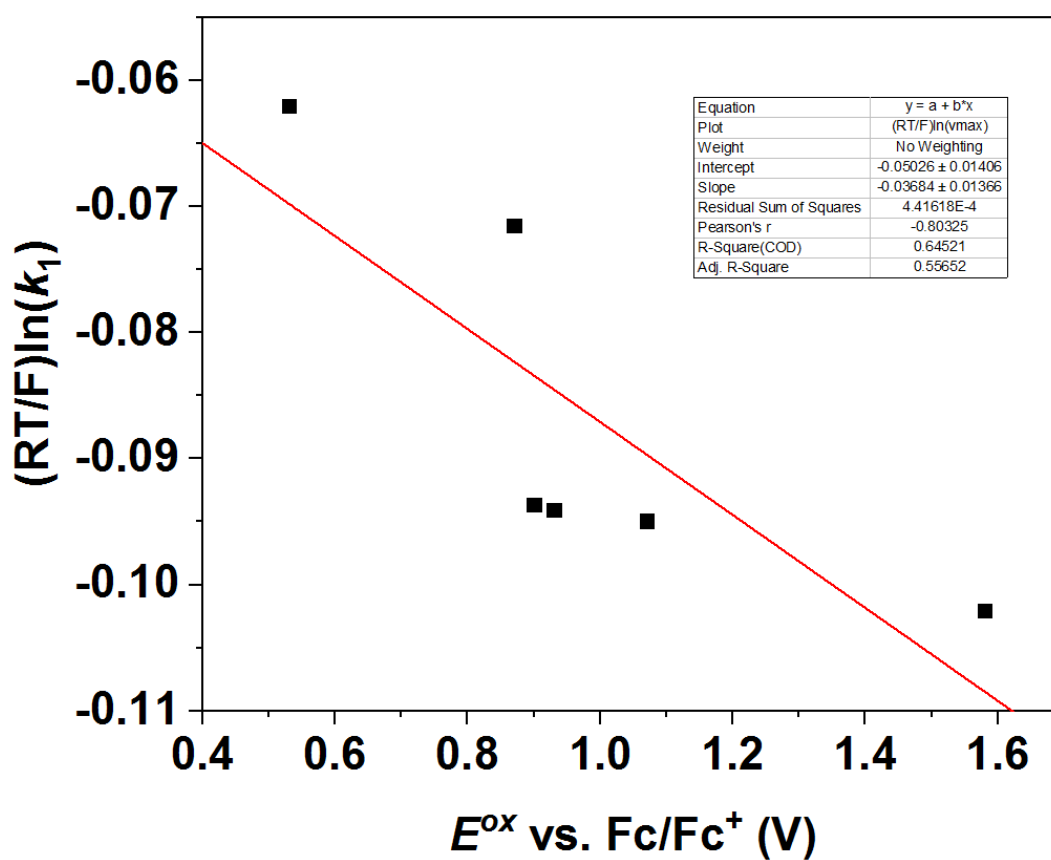


Figure A4.43: Marcus plot of $(RT/F)\ln(k_1)$ versus E_{ox} for the phenol substrates.

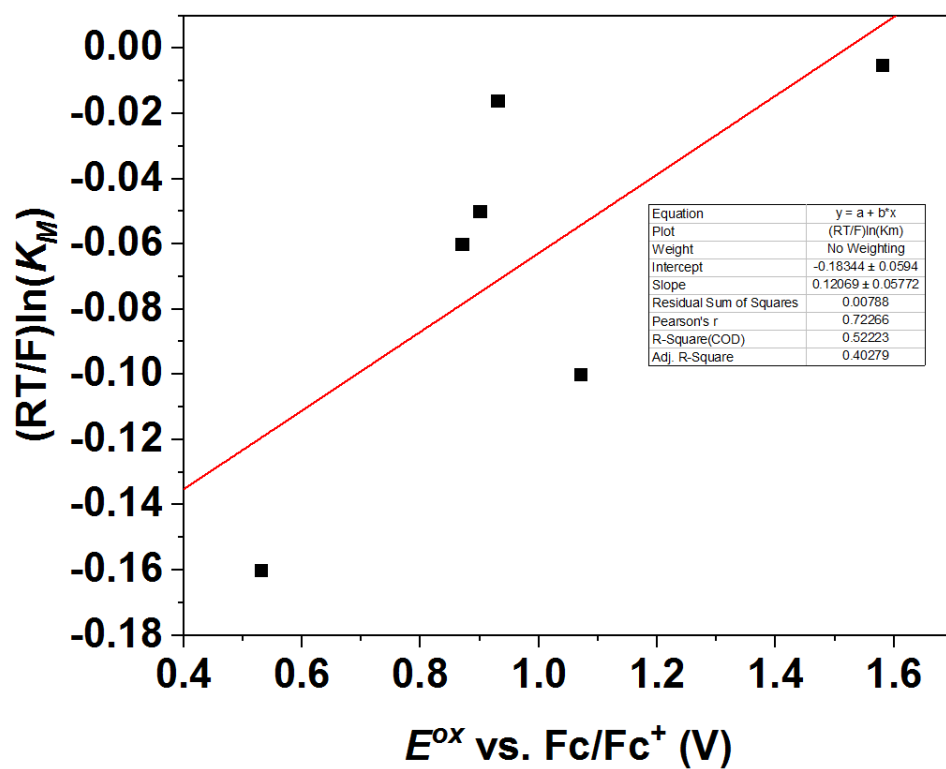


Figure A4.44: Marcus plot of $(RT/F)\ln(K_M)$ versus E_{OX} for the phenol substrates.

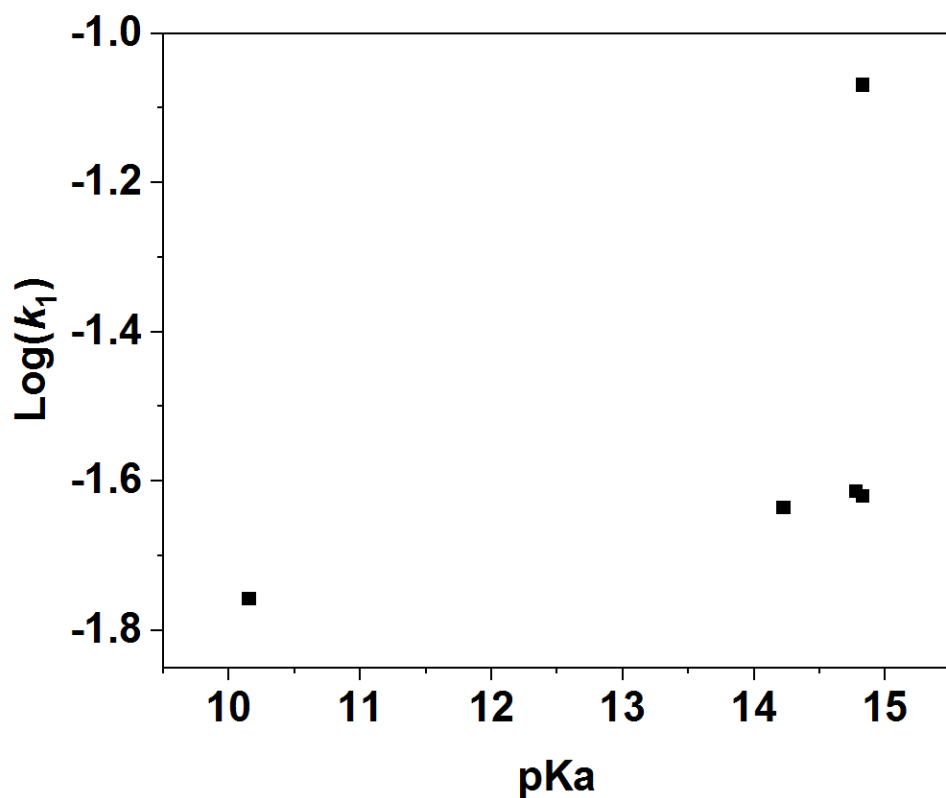


Figure A4.45: Plot of the $\log(k_1)$ versus pK_a of the phenol substrates.

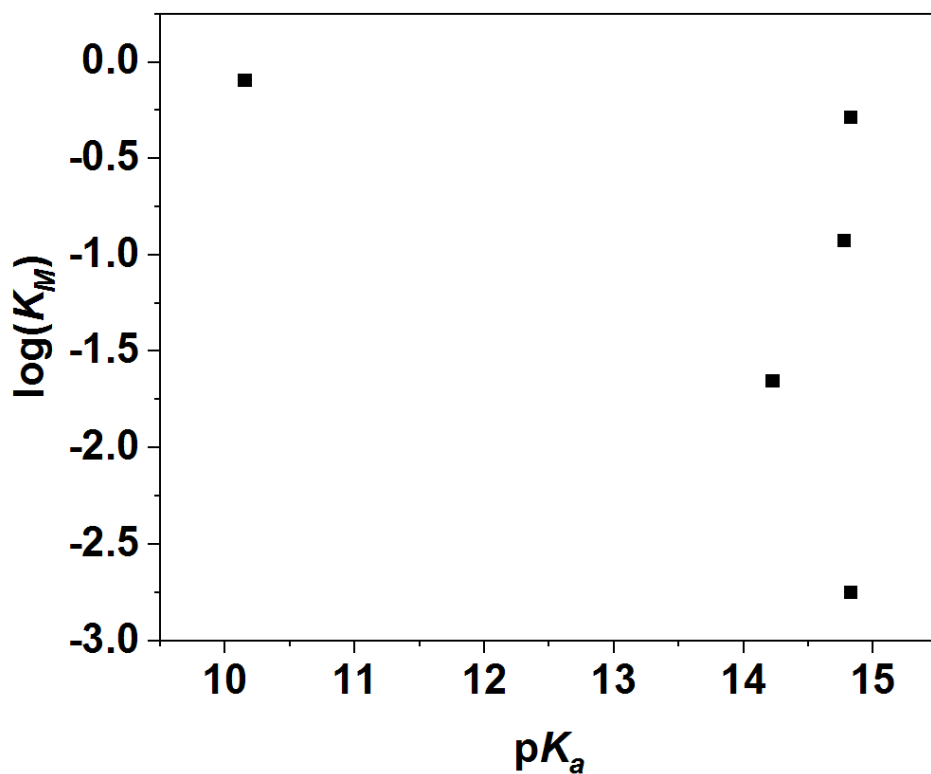


Figure A4.46: Plot of $\log(K_M)$ versus pK_a of the phenol substrates

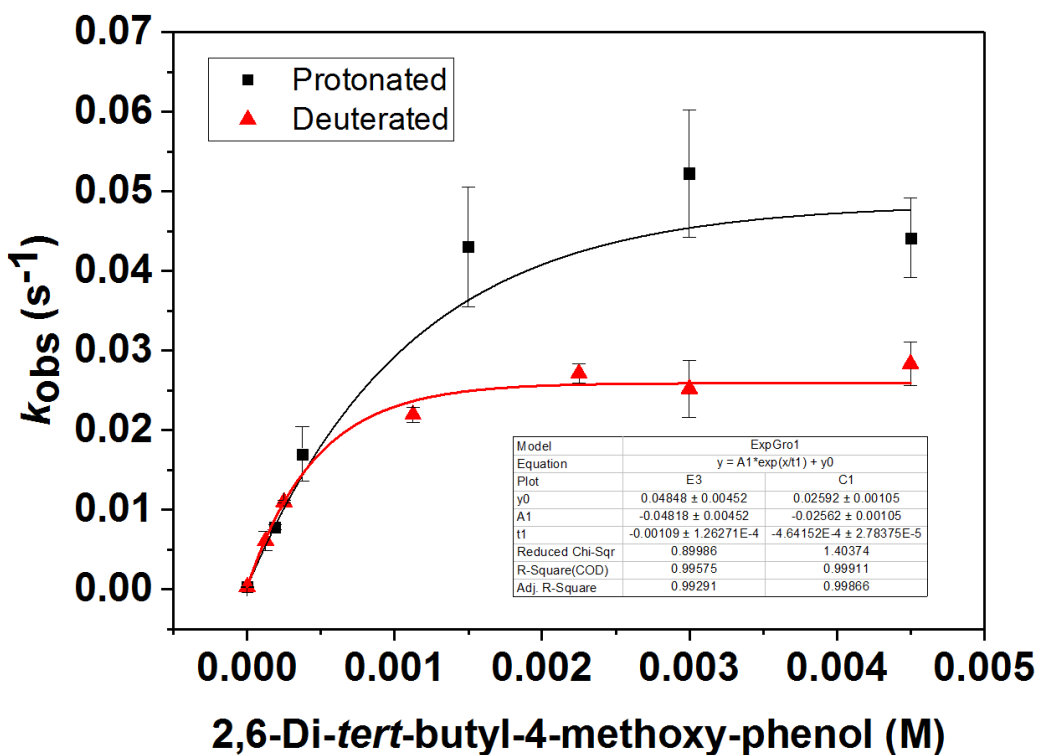


Figure A4.47: Plot of k_{obs} versus $[OH-4-OCH_3-2,6-DTBP]$ determined for the reaction of **5** and $OH-4-OCH_3-2,6-DTBP$ (black trace). Plot of k_{obs} versus $[OD-4-OCH_3-2,6-DTBP]$ determined for the reaction of **5** and $OD-4-OCH_3-2,6-DTBP$ (red trace).

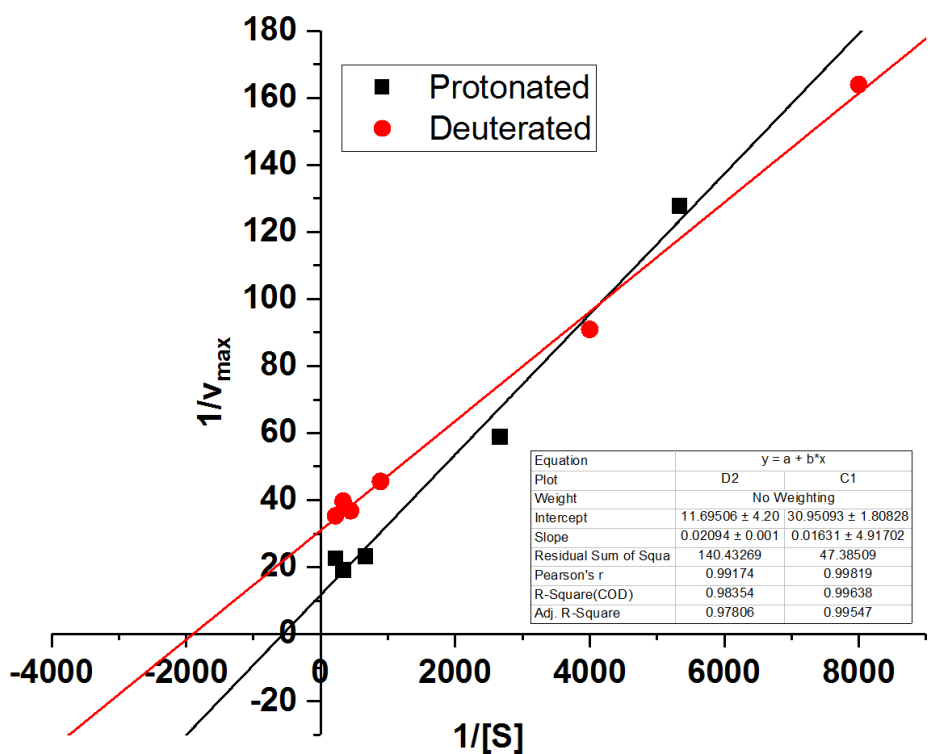


Figure A4.48: Plot of $1/k_{obs}$ versus $1/[OH-4-OCH_3-2,6-DTBP]$ determined for the reaction between **5** and *O*-H-4-OCH₃-2,6-DTBP (black trace) and plot of $1/k_{obs}$ versus $1/[OD-4-OCH_3-2,6-DTBP]$ determined for the reaction between **5** and *OD*-4-OCH₃-2,6-DTBP (red trace).

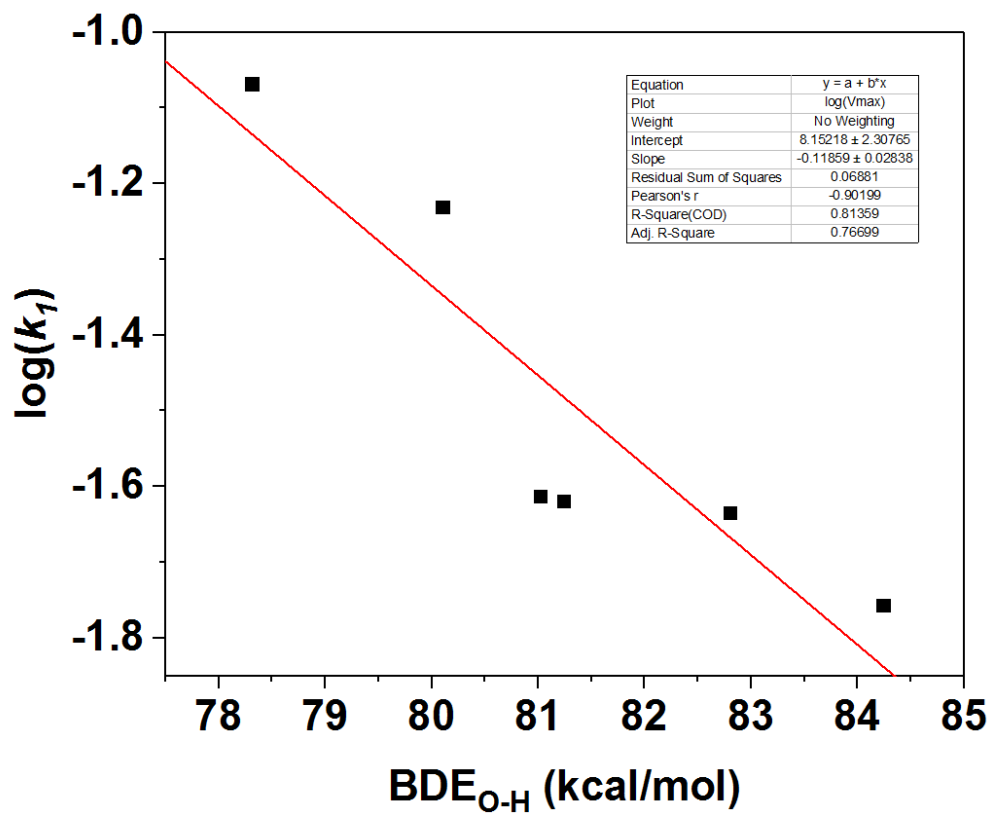


Figure A4.49: Plot of $\log(k_1)$ versus BDE_{O-H} for the phenol substrates.

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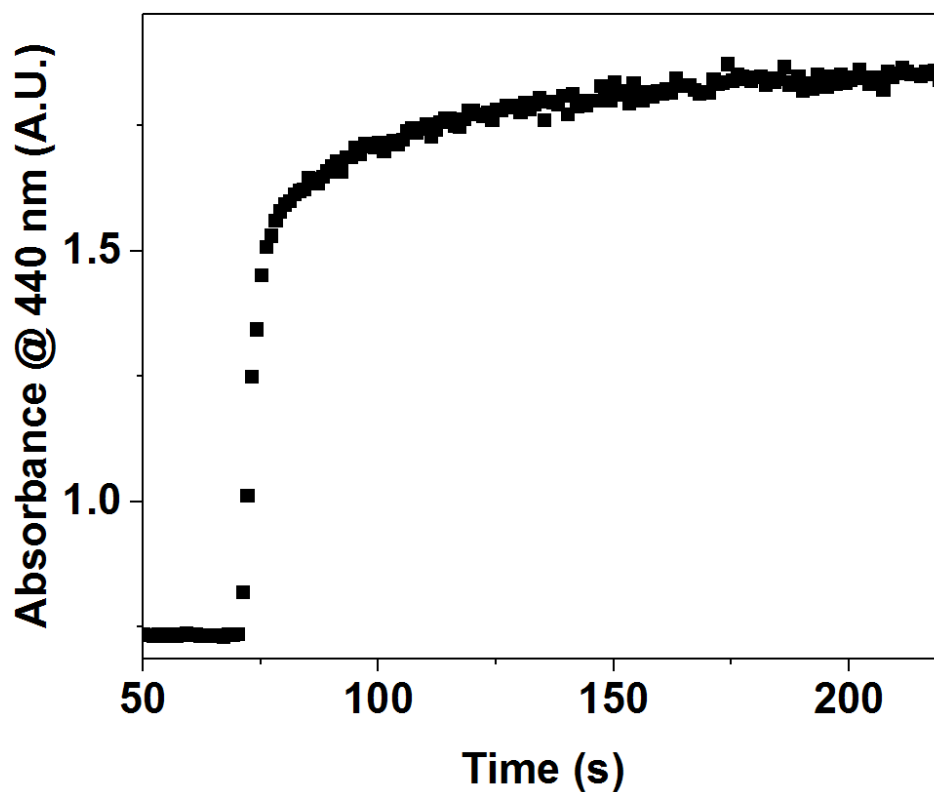


Figure A5.1: Plot of the absorbance at 440 nm during the reaction of **2** + 1 eq. $\text{Sc}(\text{OTf})_3$

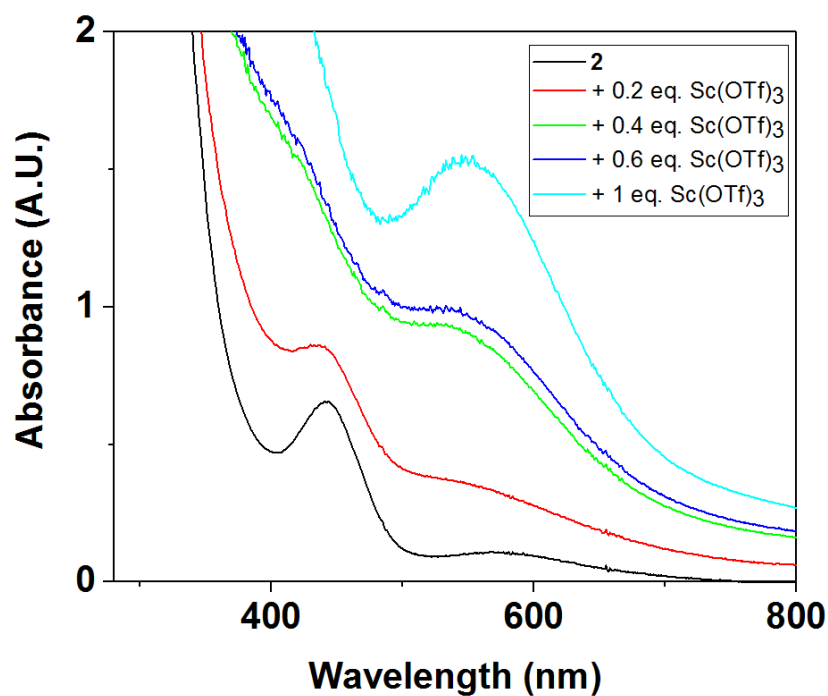


Figure A5.2: UV-vis spectrum of **2** (black trace) + 0.2 eq. $\text{Sc}(\text{OTf})_3$ (red trace), 0.4 eq. $\text{Sc}(\text{OTf})_3$ (green trace), + 0.6 eq. $\text{Sc}(\text{OTf})_3$ (blue trace), + 1 eq. $\text{Sc}(\text{OTf})_3$ (cyan trace) at -90°C in 19:MeCN/THF.

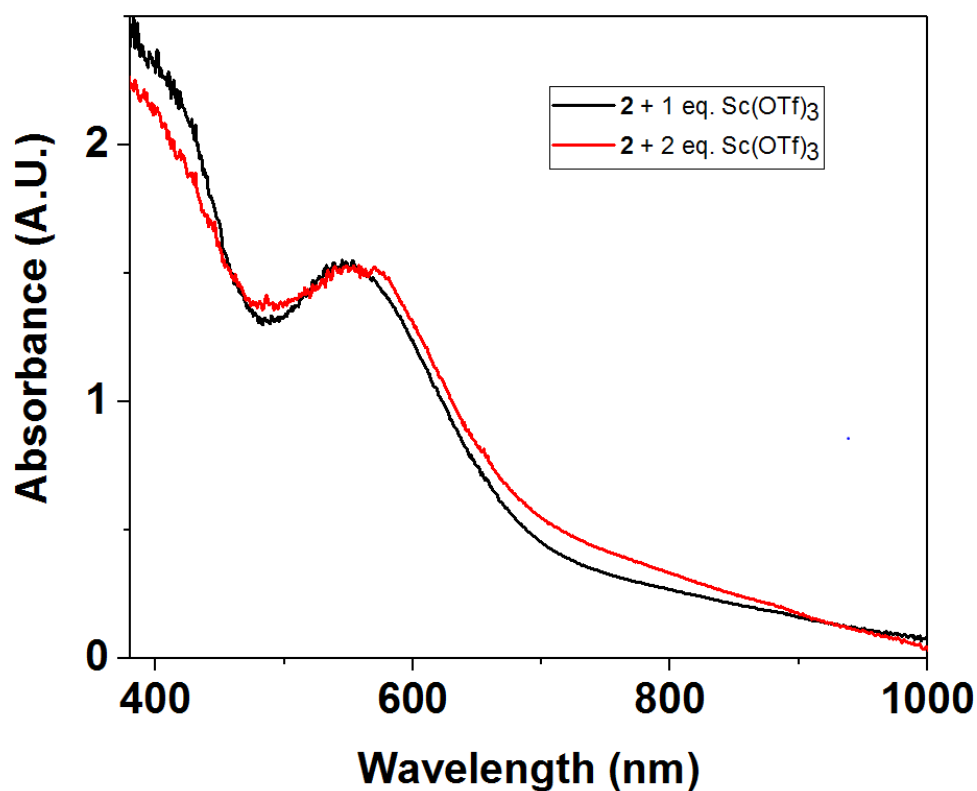


Figure A5.3: UV-vis spectra of **6** generated using 1 eq. $\text{Sc}(\text{OTf})_3$ (black trace) and 2 eq. $\text{Sc}(\text{OTf})_3$ (red trace) -90°C in 1:9 MeCN/THF.

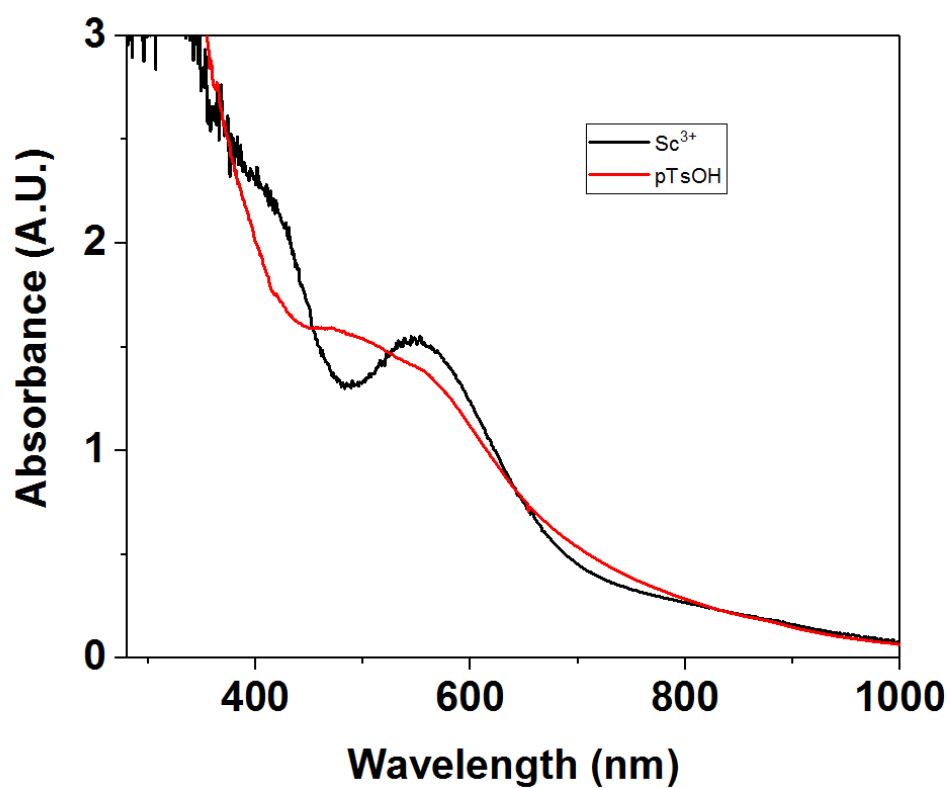


Figure A5.4: UV-vis spectra of **4** (red trace) and **6** (black trace) at -90°C in 1:9 MeCN/THF.

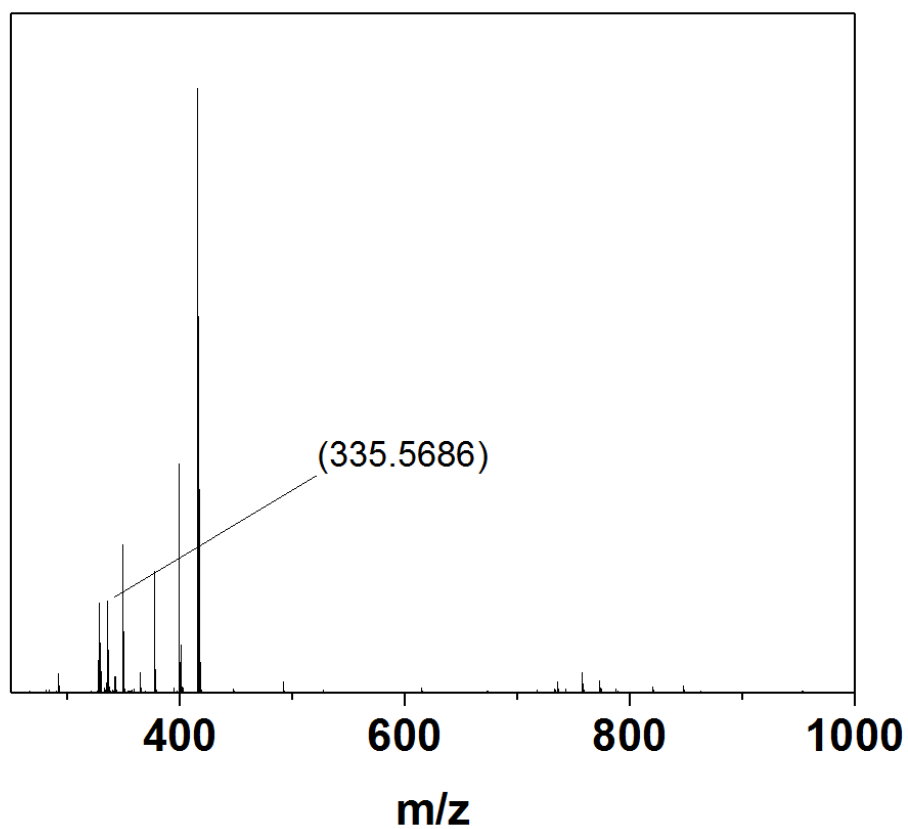


Figure A5.5: ESI-MS spectra of the post-reaction mixture of **2** + 1 eq. $\text{Sc}(\text{OTf})_3$. Highlighted peak corresponds to **6**

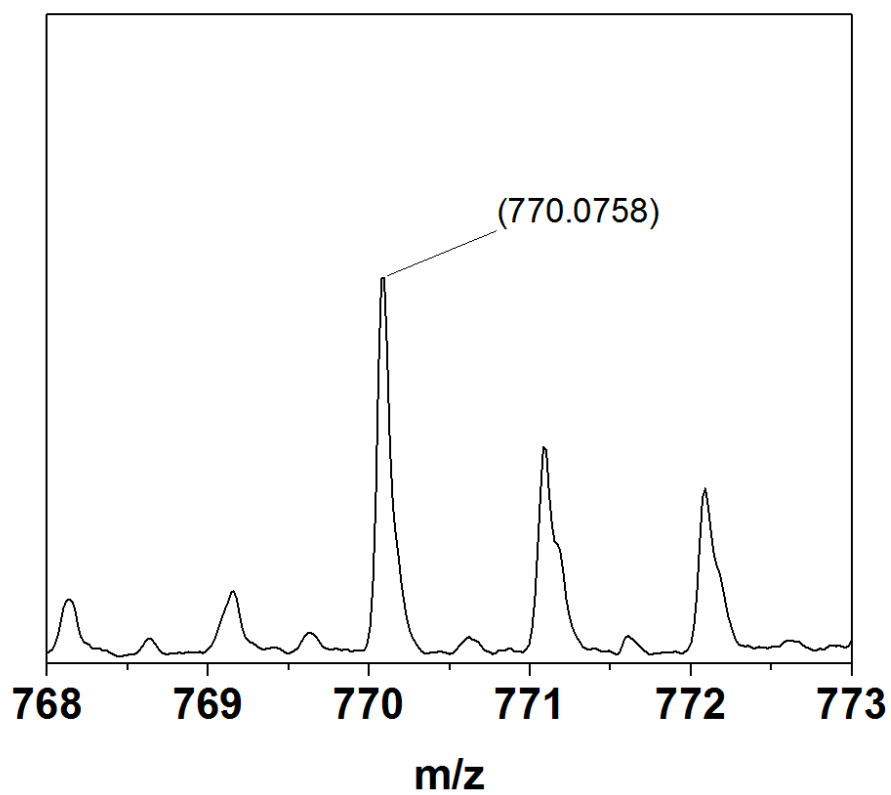


Figure A5.6: ESI-MS spectra of the post-reaction mixture of **6** expanded in the region where **2** would be expected. (**2** m/z = 770.29)

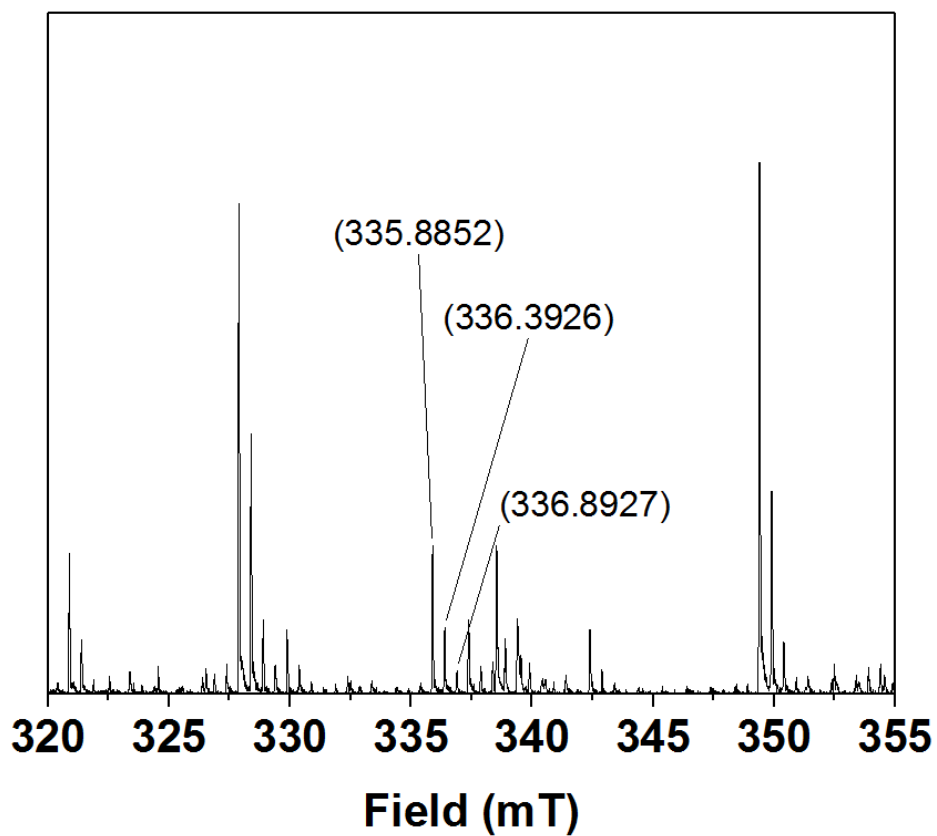


Figure A5.7: ESI-MS spectra of the post-reaction mixture of **2** expanded in the region where **6** would be expected. (**6** $m/z = 335.5682$)

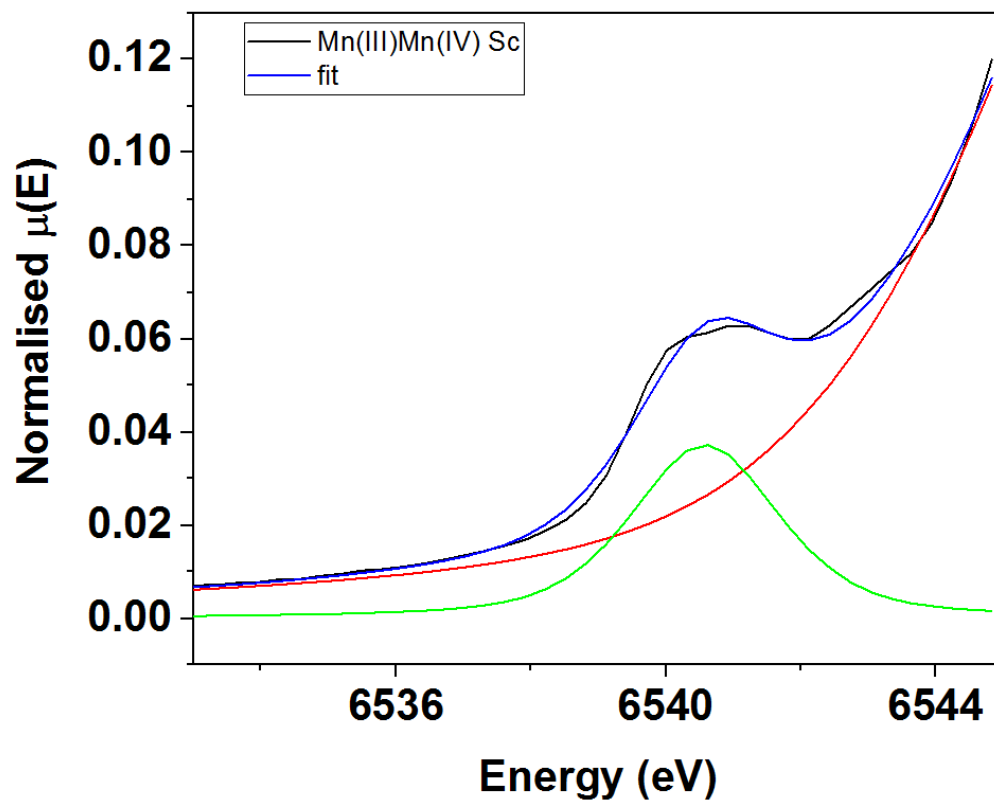


Figure A5.8: Rising edge and peak fitting of the pre-edge area of **6** with a 50:50 pseudo-Voigt function.

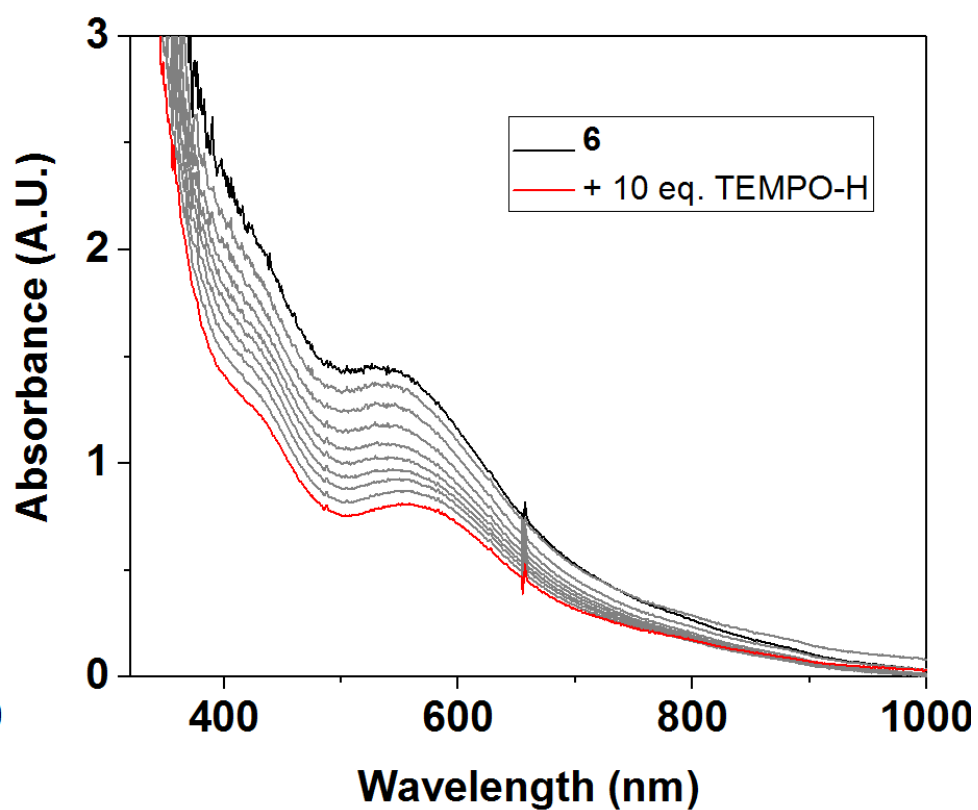


Figure 5.9: Uv-vis spectrum of the reaction of **6** (black trace) + 10 eq. TEMPO-H (red trace) at -90 °C in 1:9 MeCN/THF.

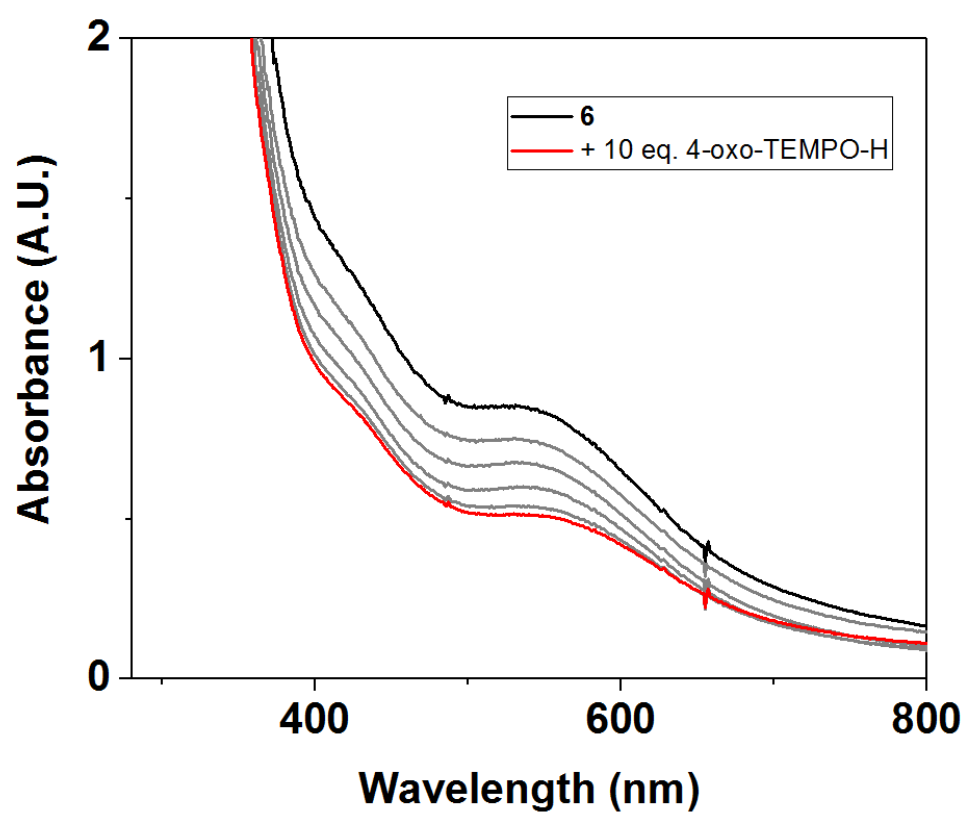


Figure A5.10: Uv-vis spectrum of the reaction of **6** (black trace) + 10 eq. 4-oxo-TEMPO-H (red trace) at -90 °C in 1:9 MeCN/THF.

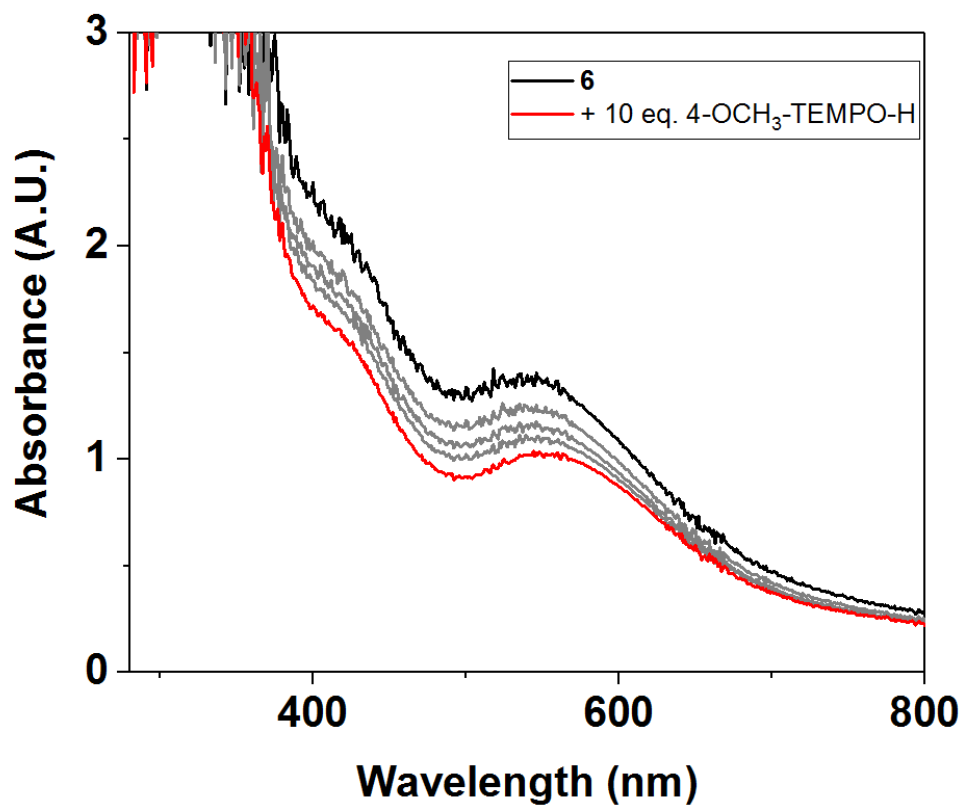


Figure A5.11: Uv-vis spectrum of the reaction of **6** (black trace) + 10 eq. 4-OCH₃-TEMPO-H (red trace) at -90 °C in 1:9 MeCN/THF.

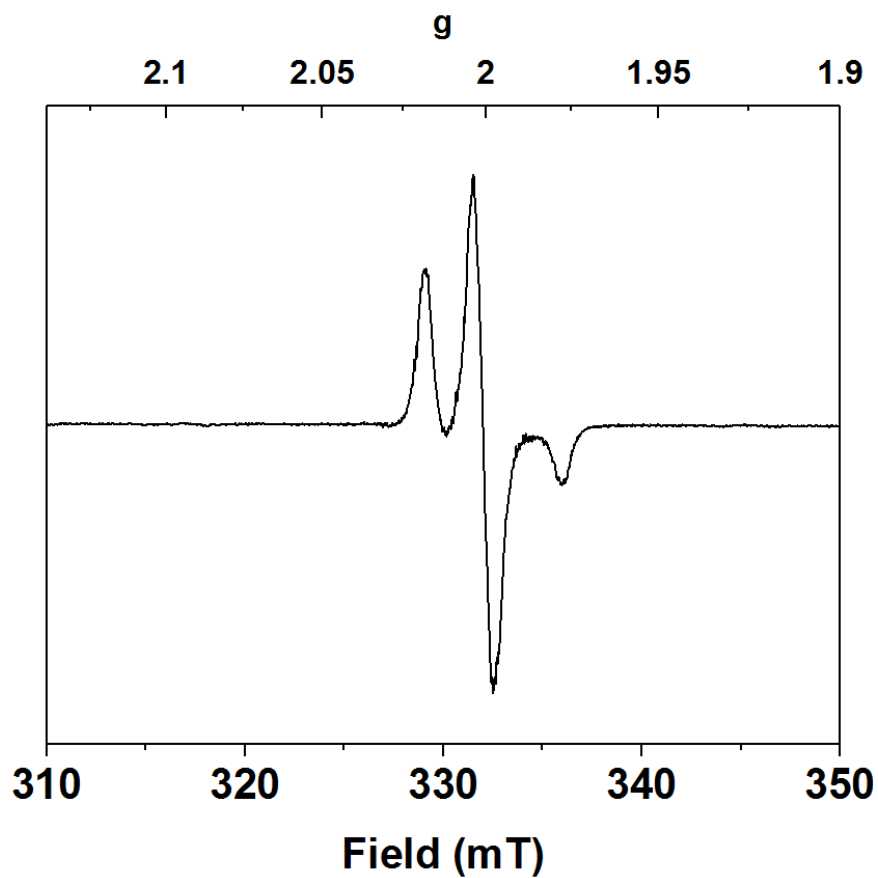


Figure A5.12: EPR spectrum of the post-reaction mixture of **6** + 10 eq. 4-oxo-TEMPO-H

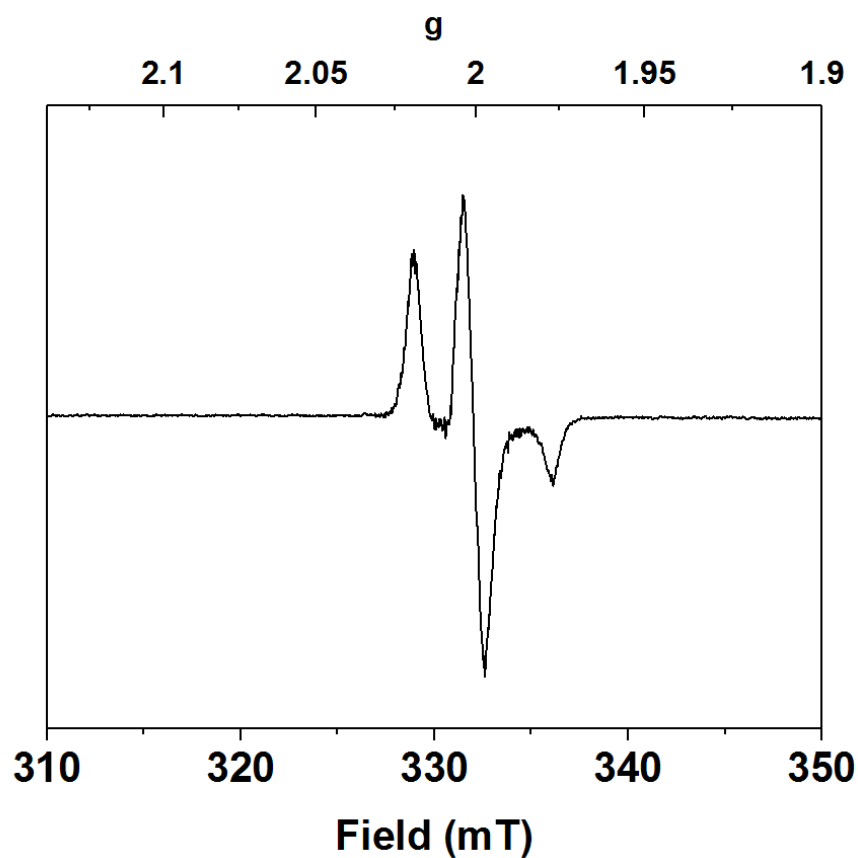


Figure A5.13: EPR spectrum of the post-reaction mixture of **6** + 10 eq. 4-OCH₃-2,6-TEMPO-H

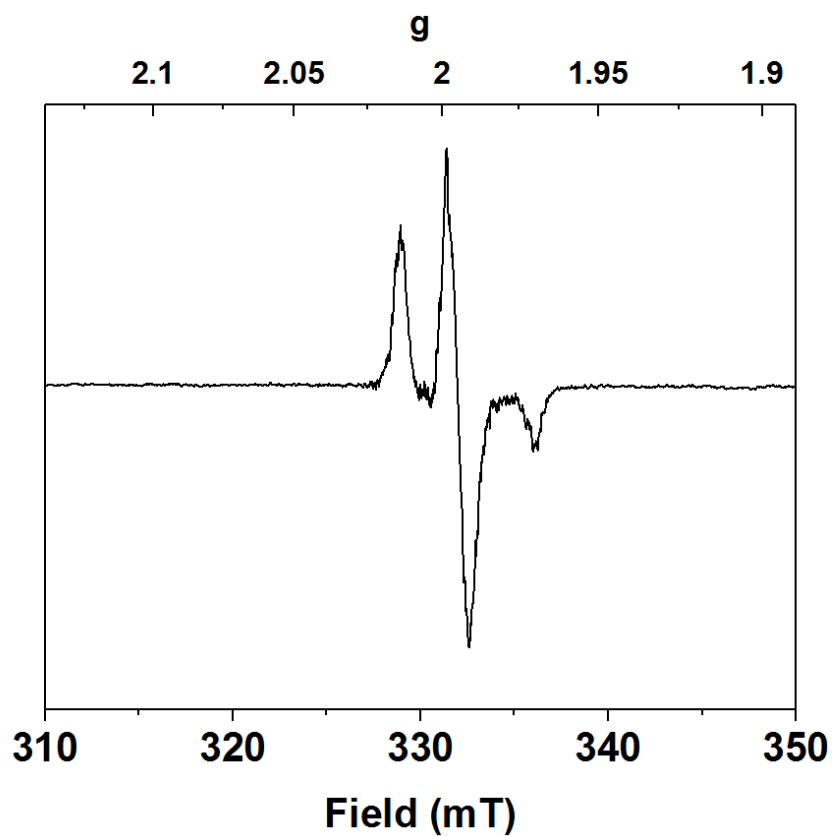


Figure A5.14: EPR spectrum of the post-reaction mixture of **6** + 10 eq. TEMPO-H

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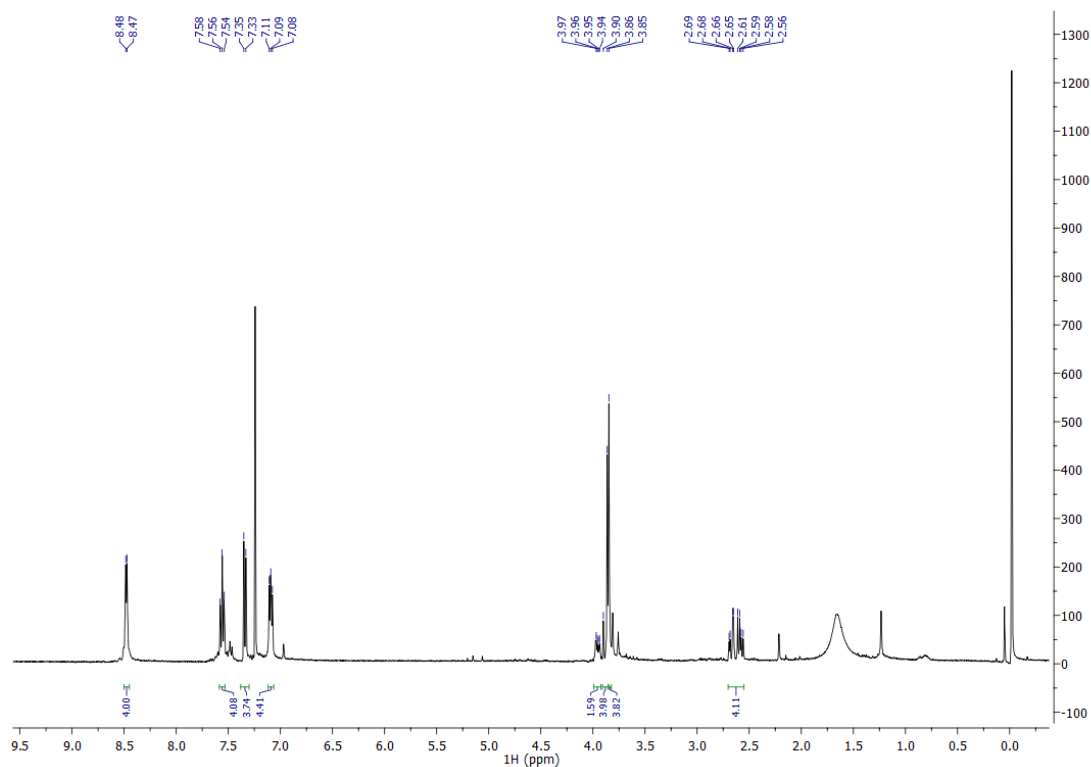


Figure A6.1: NMR spectrum of TPD ligand in CDCl_3

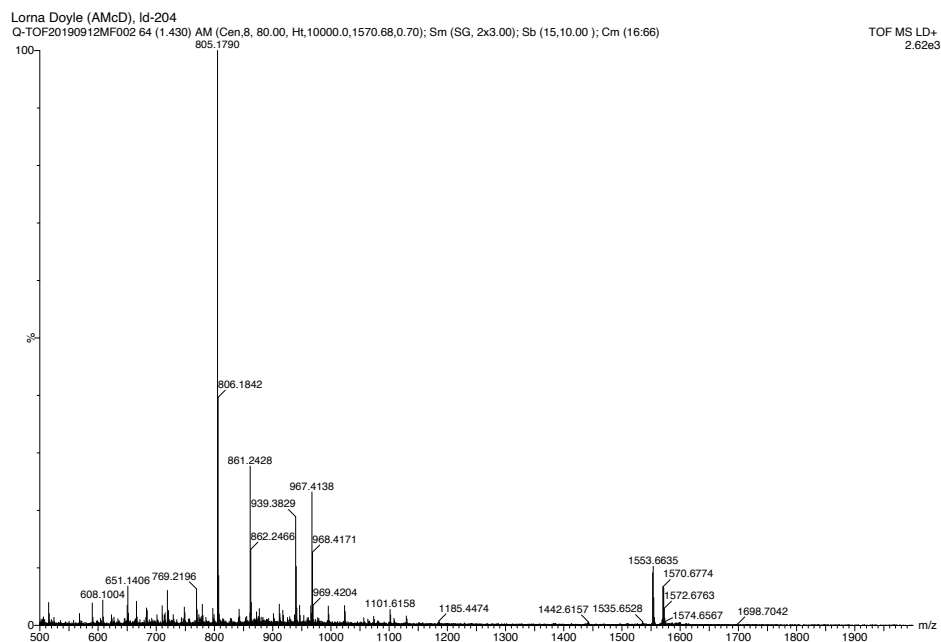


Figure A6.2: Mass spectrum of $[\text{Mn}^{\text{II}}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2](\text{BPh}_4)$. $m/z = 805.1790 = [\text{Mn}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2]^+$

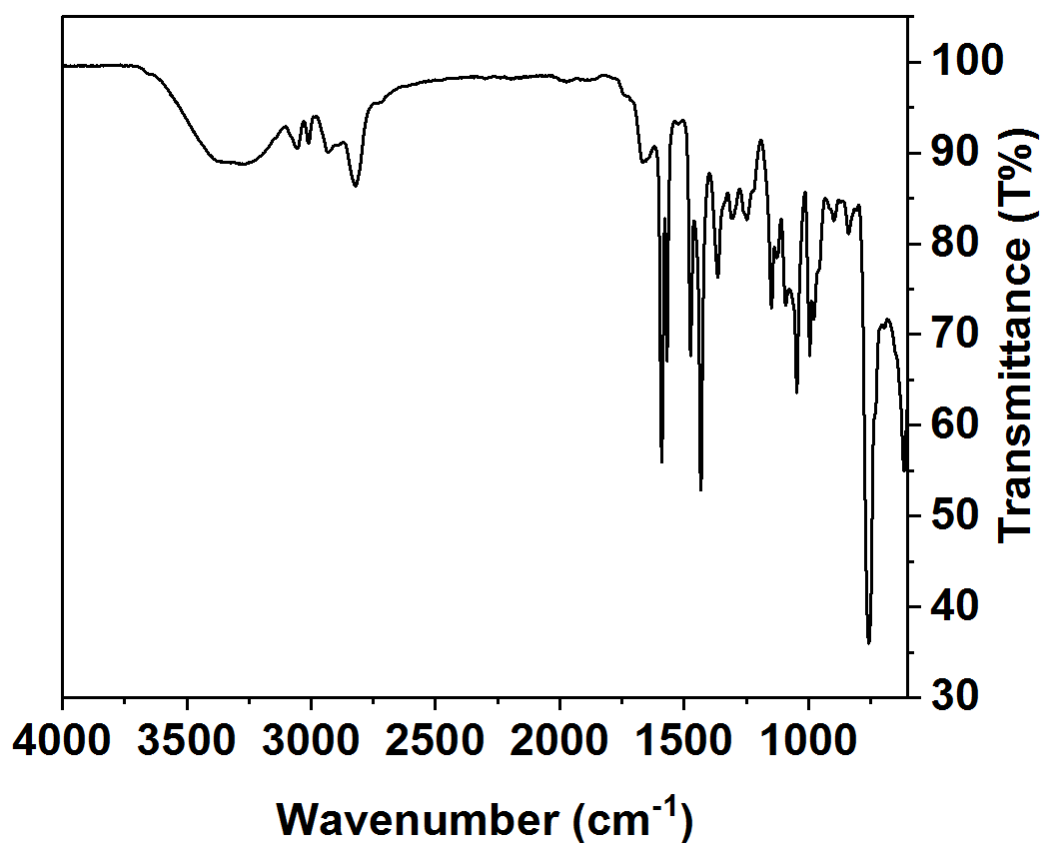


Figure A6.3: IR spectrum of TPDP ligand

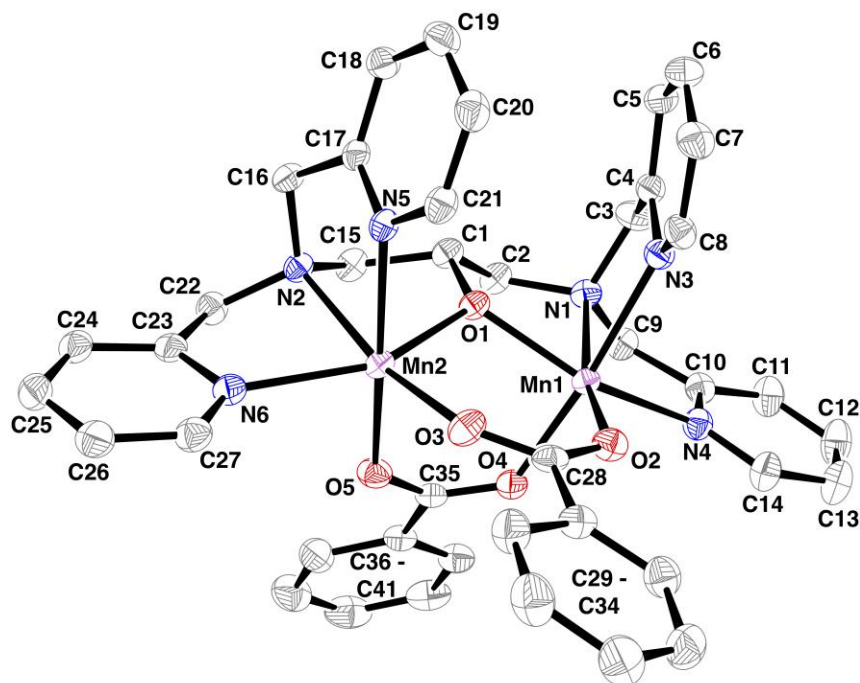


Figure A6.4: Crystals of $[\text{Mn}_2(\text{TPDP})(\text{O}_2\text{CC}_6\text{H}_5)_2]\text{BPh}_4 \cdot 2\text{MeCN}$ were obtained from MeCN solution stored at -28°C . The anion, solvent molecules and hydrogen atoms are omitted for clarity. Ellipsoids are depicted at 50% probability level.

Table A6.5: Crystal data and structure refinement for of $[Mn^{II}_2(TPDP)(O_2CC_6H_5)_2](BPh_4)$

Identification Code	RGDRBI014P201
Empirical Formulae	C ₆₉ H ₆₅ B Mn ₂ N ₈ O ₅
Formula Weight	1206.98
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal System	Triclinic
Space Group	P-1
Unit Cell Dimensions	a = 8.8517(7) Å b = 17.5737(15) Å c = 20.1124(16) Å α = 96.904(3)° β = 92.863(3)° γ = 103.317(3)°
Volume	3012.6(4) Å ³
Z	2
Density (calculated)	1.331 Mg/m ³
Absorption Coefficient	0.478 mm ⁻¹
F(000)	1260
Crystal Size	0.321 x 0.272 x 0.226 mm ³
Theta range for data collection	1.681 to 27.522°
Index ranges	-11 ≤ h ≤ 10, -22 ≤ k ≤ 22, -26 ≤ l ≤ 26
Reflections collected	66765
Independent reflections	13838 [R(int) = 0.0282]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7456 and 0.6629
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	13838 / 0 / 768
Goodness-of-fit on F ²	1.029
Final R indices [I > 2σ(I)]	R ₁ = 0.0359, wR ₂ = 0.0920
R indices (all data)	R ₁ = 0.0451, wR ₂ = 0.0960
Largest diff. peak and hole	0.409 and -0.275 e.Å ⁻³

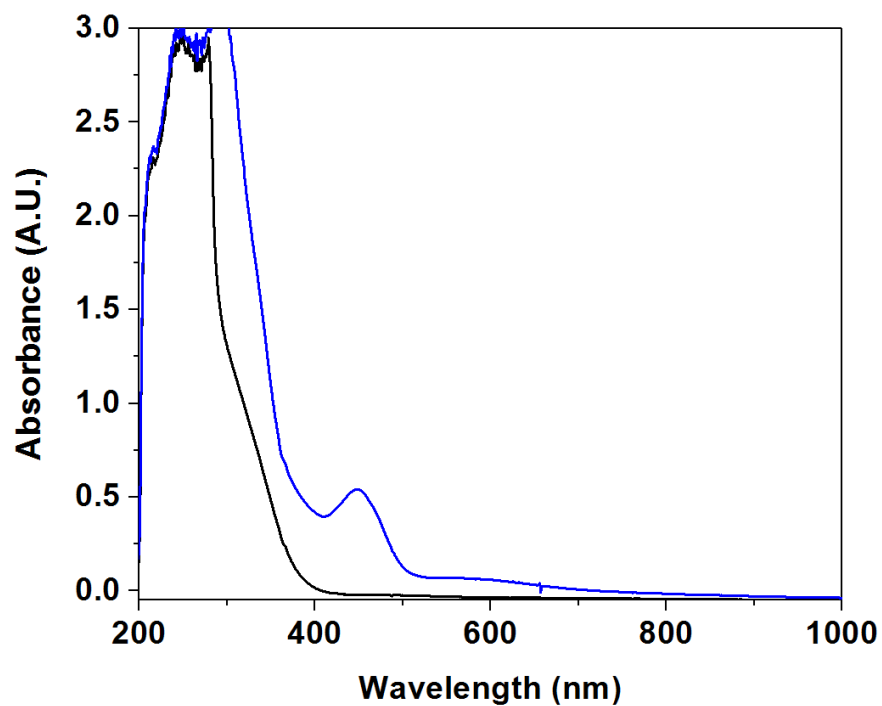


Figure A6.6: UV-Vis spectra of the reaction of 1.5 mM **7** (black trace) +1 eq. KO₂ to form **8** (blue trace) in 1:9 MeCN/THF at -90 °C.

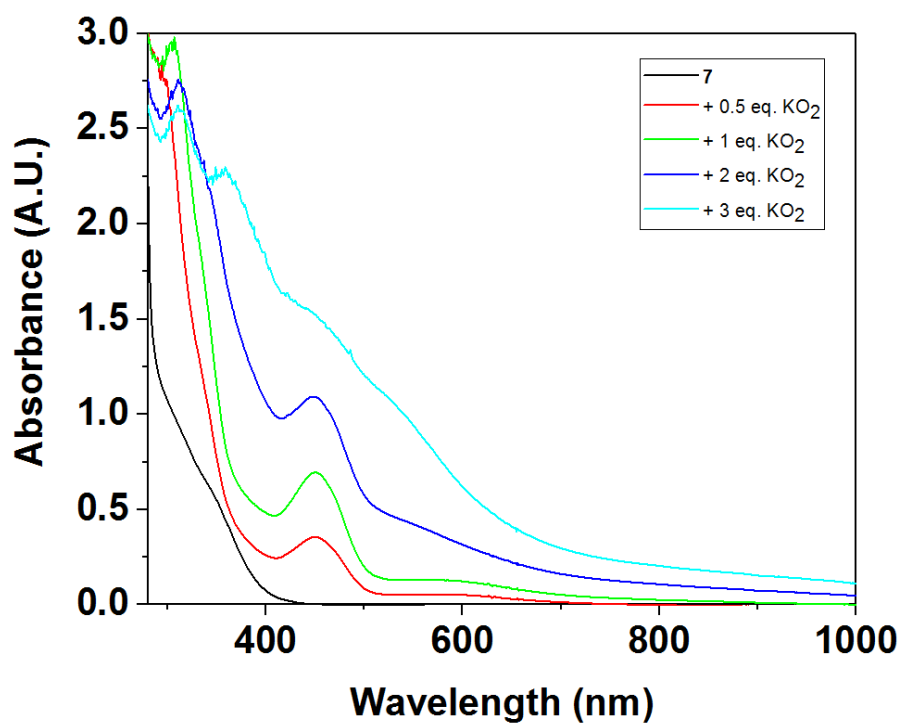


Figure A6.7: UV-Vis spectra of the reaction of 1.5 mM **7** (black trace) +0.5 eq.(red trace), + 1 eq. (green trace), +2 eq. (blue trace), and 3 eq. (cyan trace) KO₂ in 1:9 MeCN/THF at -90 °C.

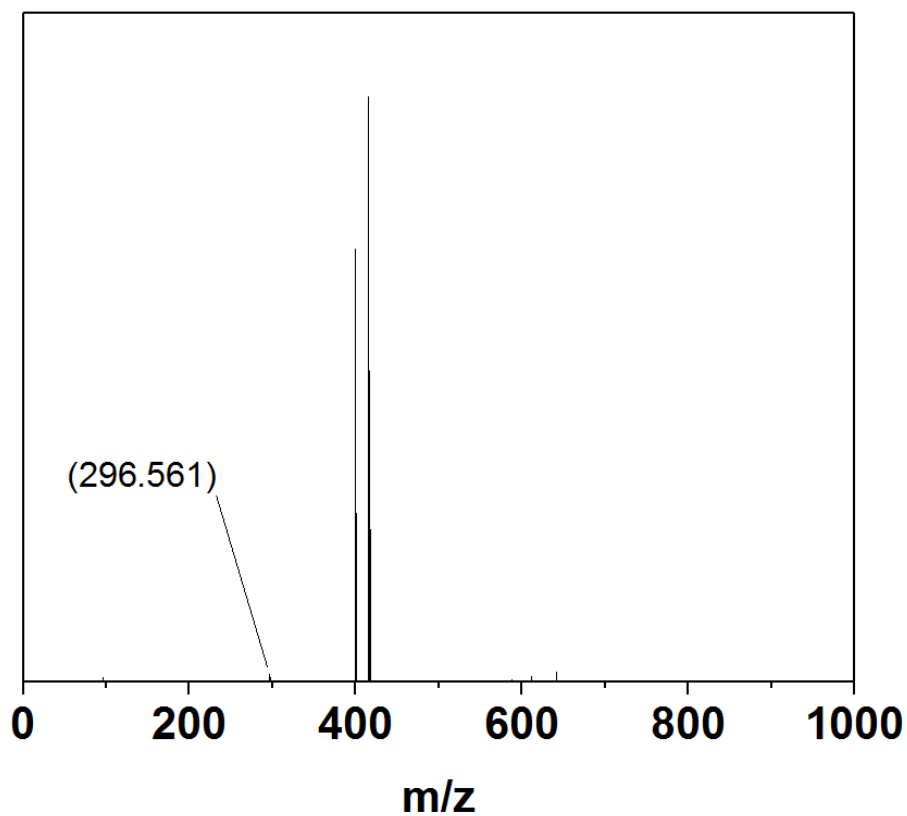


Figure A6.8: ESI-MS spectra of the post-reaction mixture of **7** + 1 eq. KO_2 . Highlighted peak corresponds to **8**

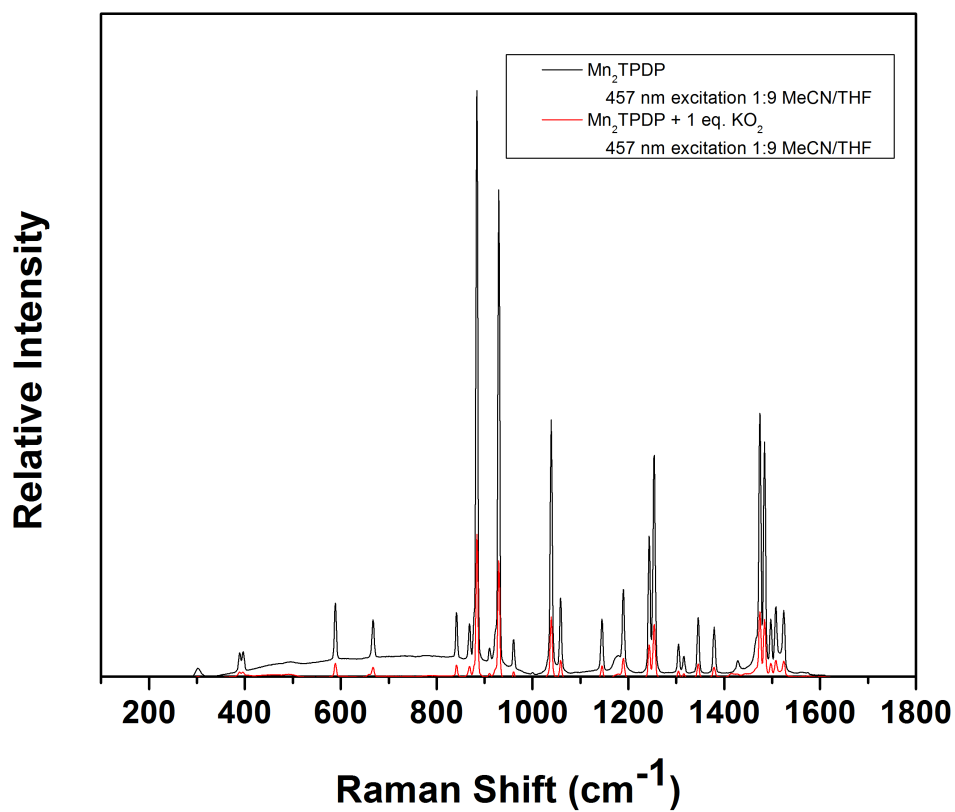


Figure A6.9: Resonance Raman spectra of **7** (black trace) and **8** (red trace) in 1:9 MeCN/THF measured at 77 K and excited at 457 nm.

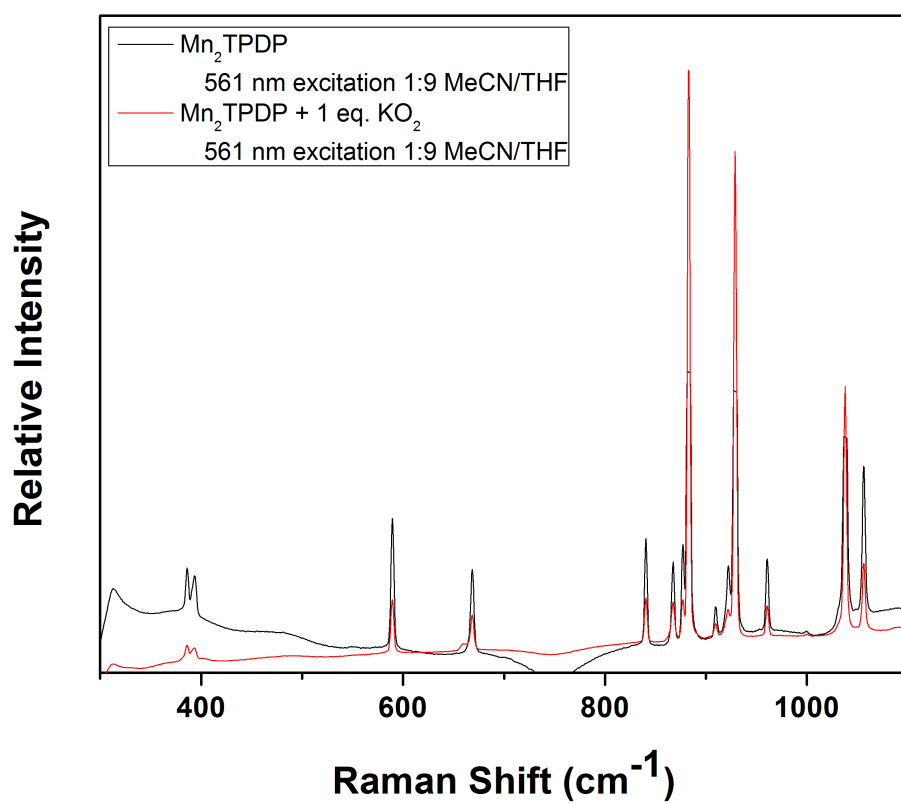


Figure A6.10: Resonance Raman spectra of **7** (black trace) and **8** (red trace) in 1:9 MeCN/THF measured at 77 K and excited at 561 nm.

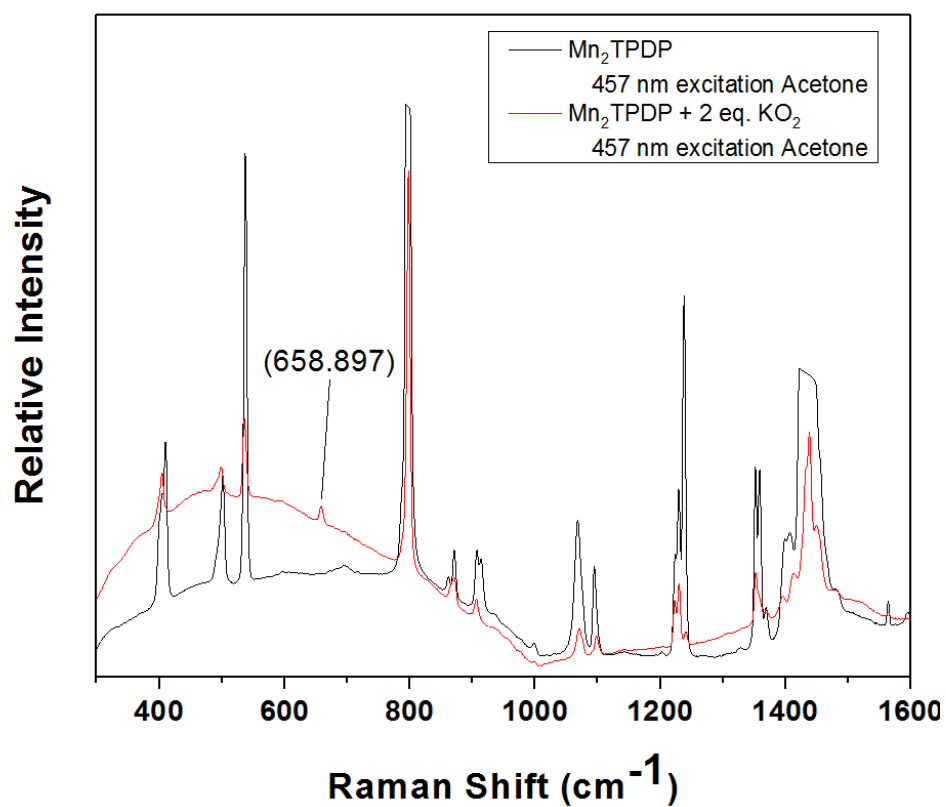


Figure A6.11: Resonance Raman spectra of **7** (black trace) and **8** (red trace) in acetone measured at 77 K and excited at 457 nm.

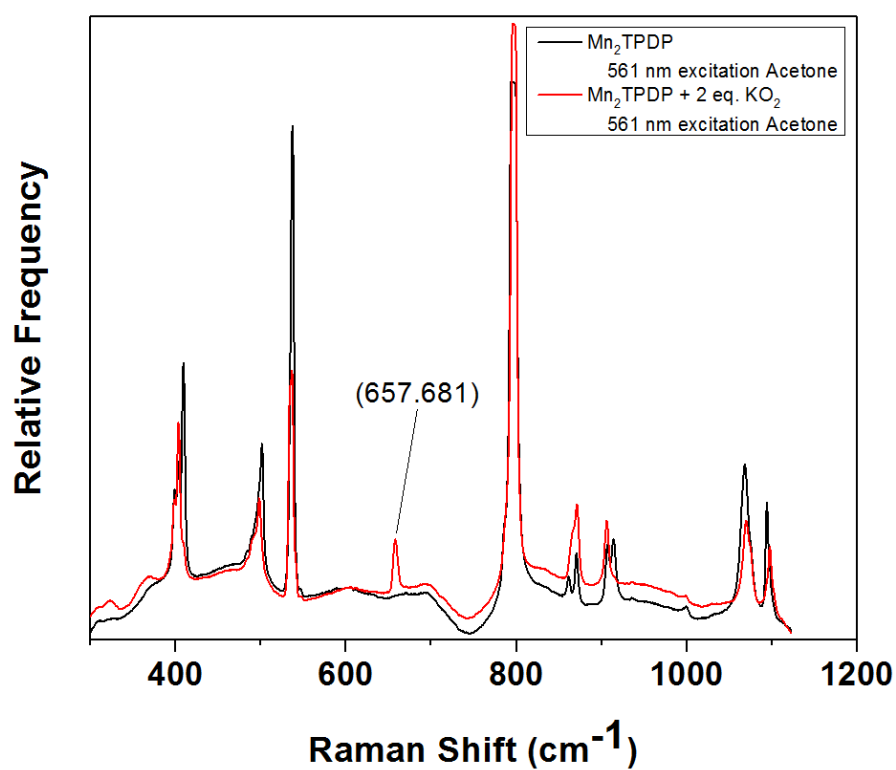


Figure A6.12: Resonance Raman spectra of 7 (black trace) and 8 (red trace) in acetone measured at 77 K and excited at 561 nm.

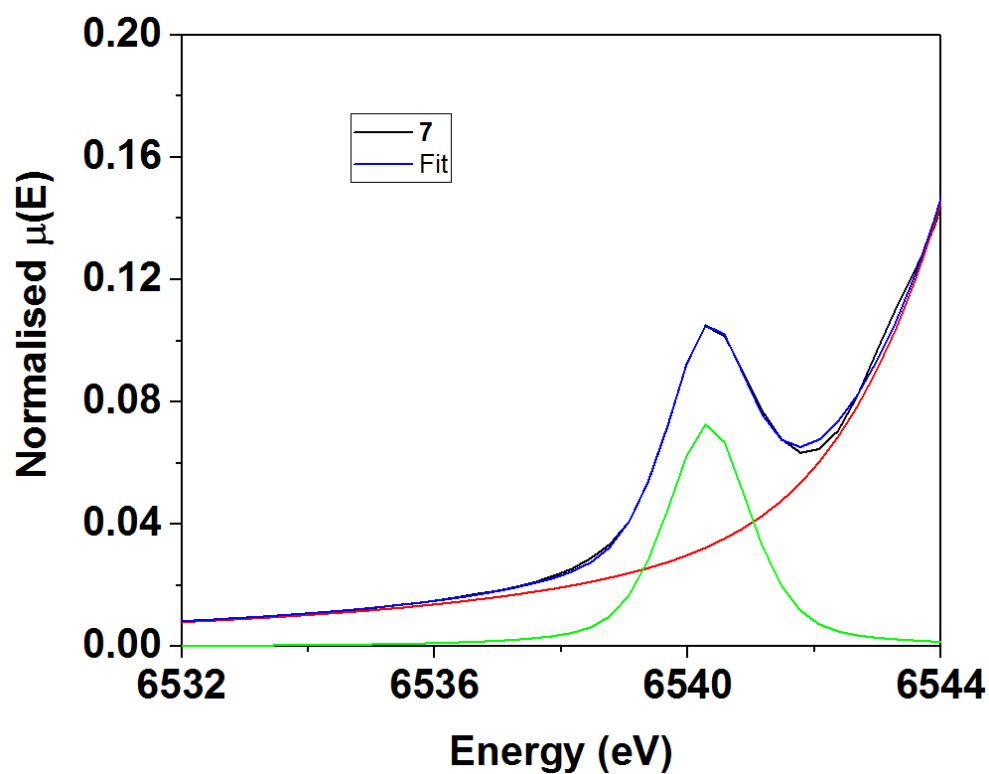


Figure A6.13: Rising edge and peak fitting of the pre-edge area of 7 with a 50:50 pseudo-Voigt function.

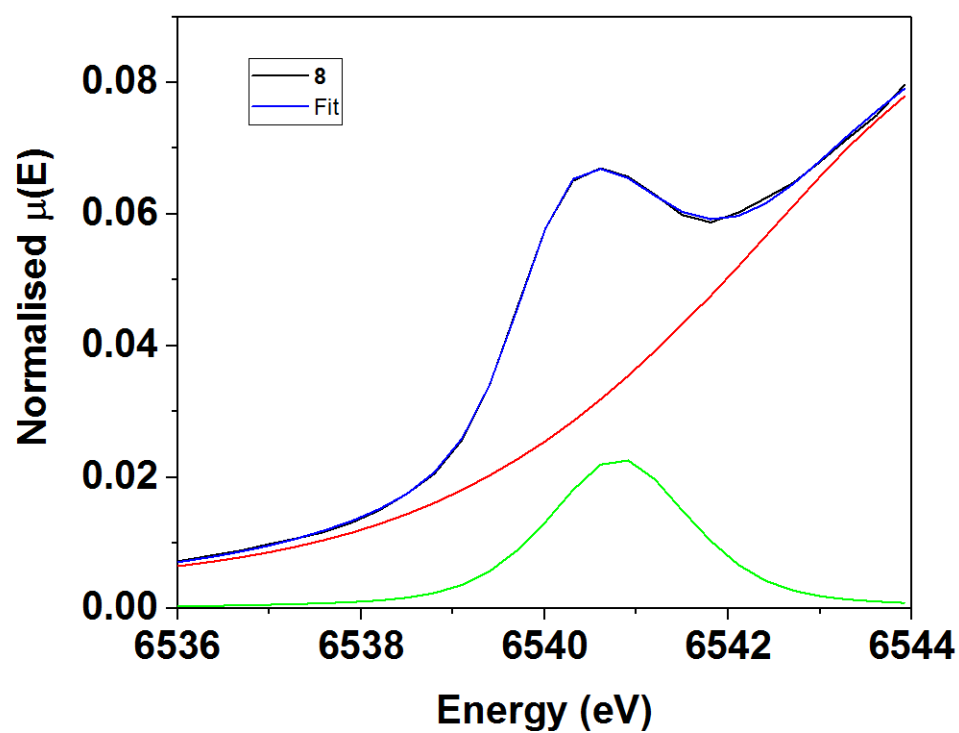


Figure A6.14: Rising edge and peak fitting of the pre-edge area of **8** with a 50:50 pseudo-Voigt function.

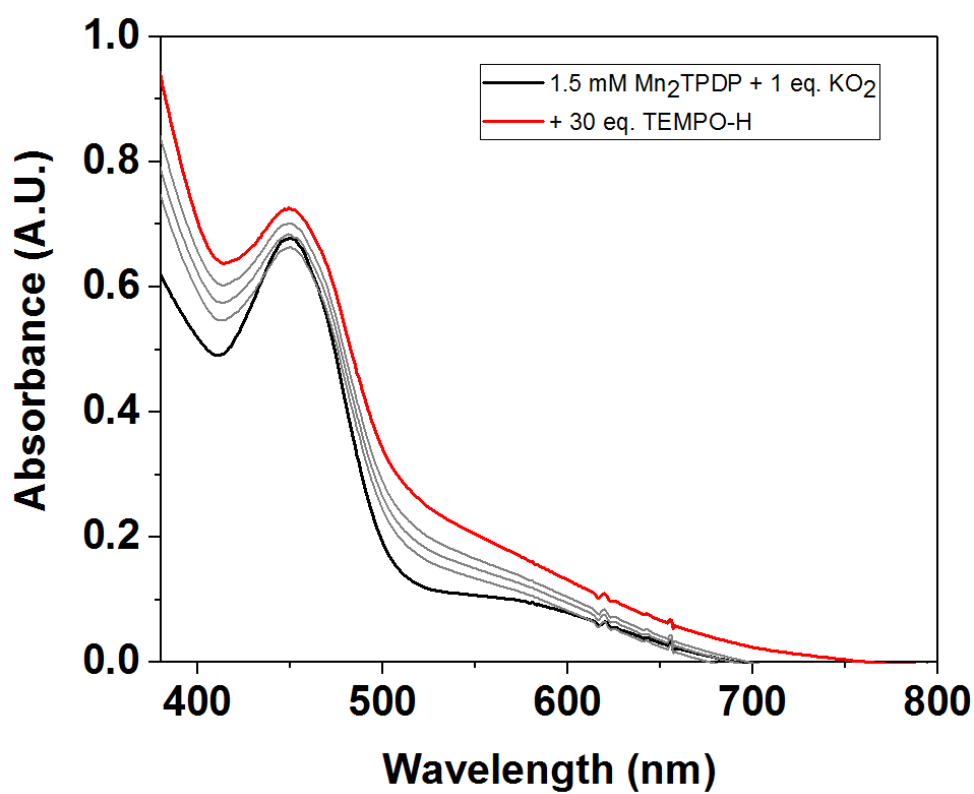


Figure A6.15: UV-vis spectra of the reaction of **8** + 30 eq. TEMPO-H in 1:9 MeCN/THF at -90 °C.

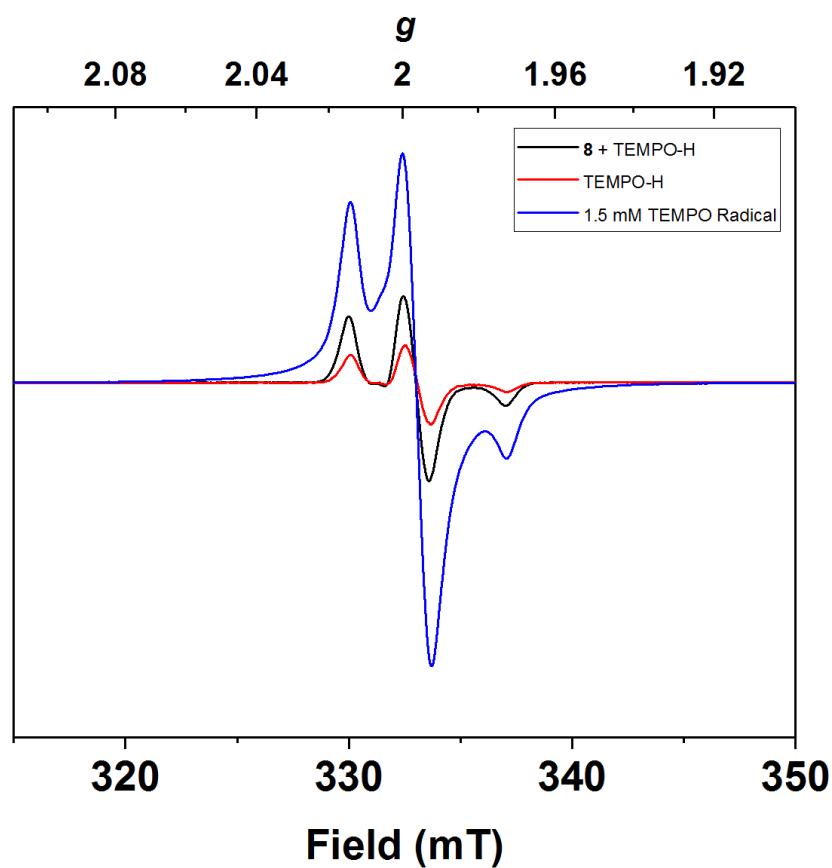


Figure A6.16: EPR spectra of **8** + 30 eq. TEMPO-H (black trace), 30 eq. of TEMPO-H (red trace), 1.5 mM TEMPO radical (blue trace). (77K, 9.65 GHz, 0.2 mW microwave power).

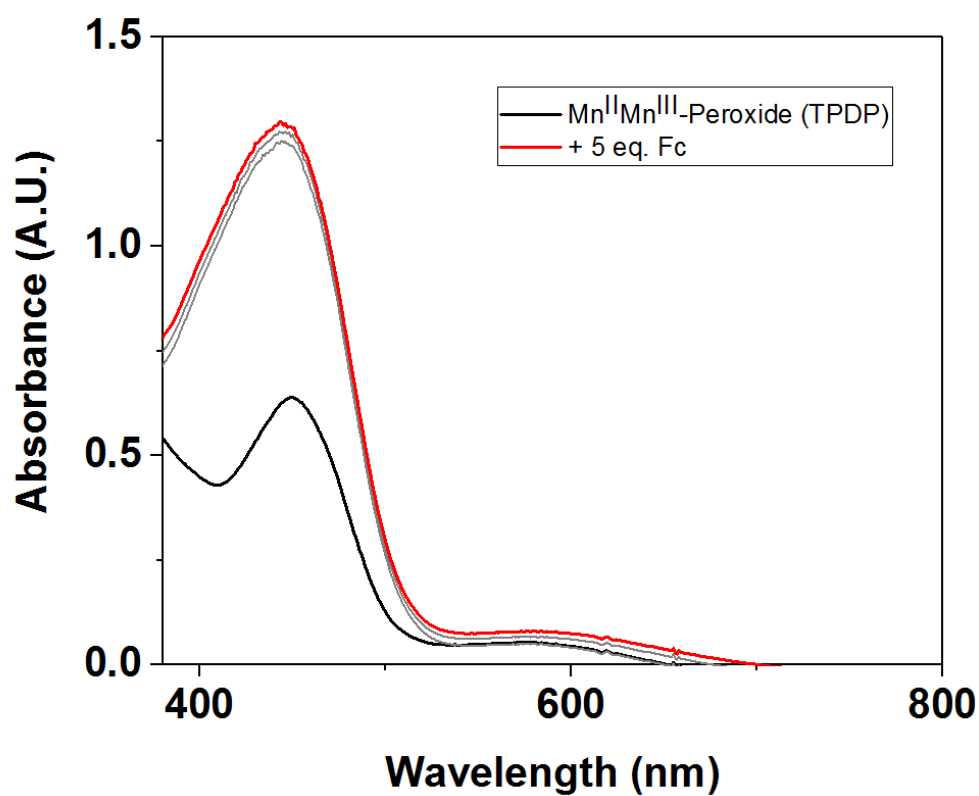


Figure A6.17: UV-vis spectra of the reaction of **8** + 5 eq. ferrocene in 1:9 MeCN/THF at -90 °C.

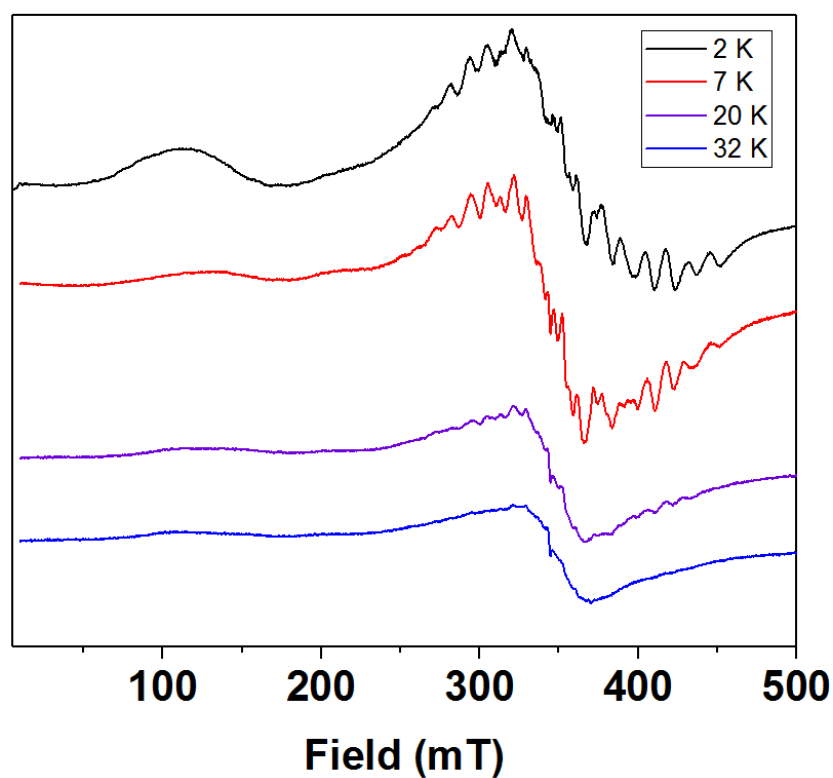


Figure A6.18: Perpendicular X-Band EPR spectra of **9** recorded at 2 K (black), 7 K (red), 20 K (purple), and 32 K (blue) (9.65 GHz, 0.2 mW microwave power).

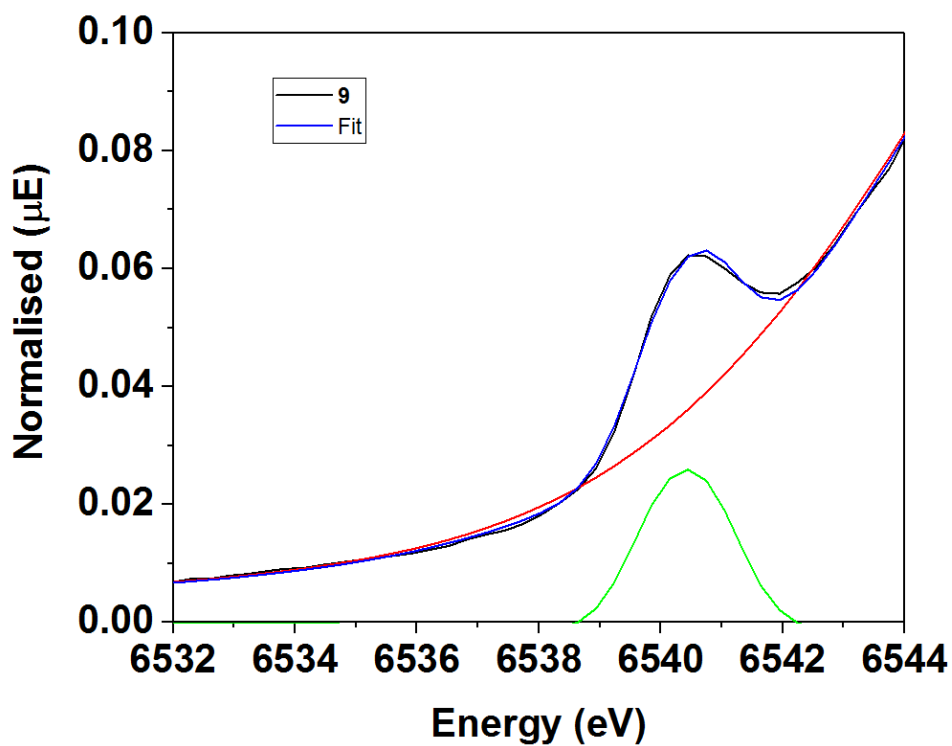


Figure A6.19: Rising edge and peak fitting of the pre-edge area of **9** with a 50:50 pseudo-Voigt function.

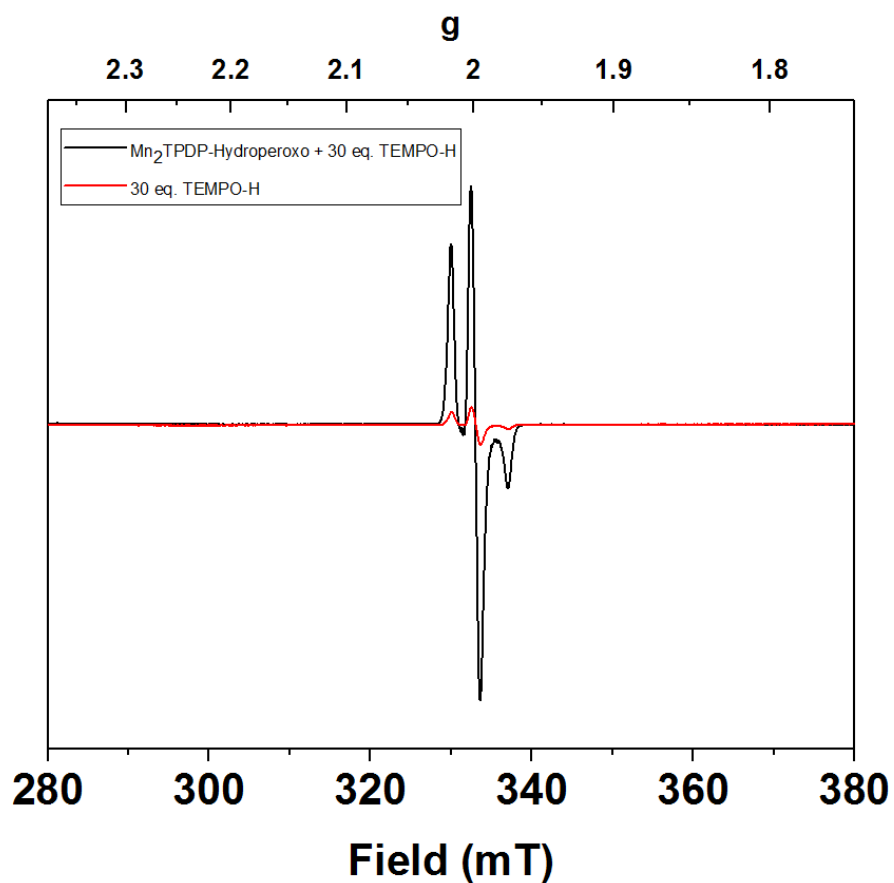


Figure A6.20: Black trace: Perpendicular X-band EPR spectrum of the post-reaction mixture of **9** + 30 eq. TEMPO-H (9.34 GHz, 2.00 mW microwave power) Red trace: Perpendicular X-band EPR spectrum control reaction of 30 eq. TEMPO-H (9.34 GHz, 2.00 mW microwave power).

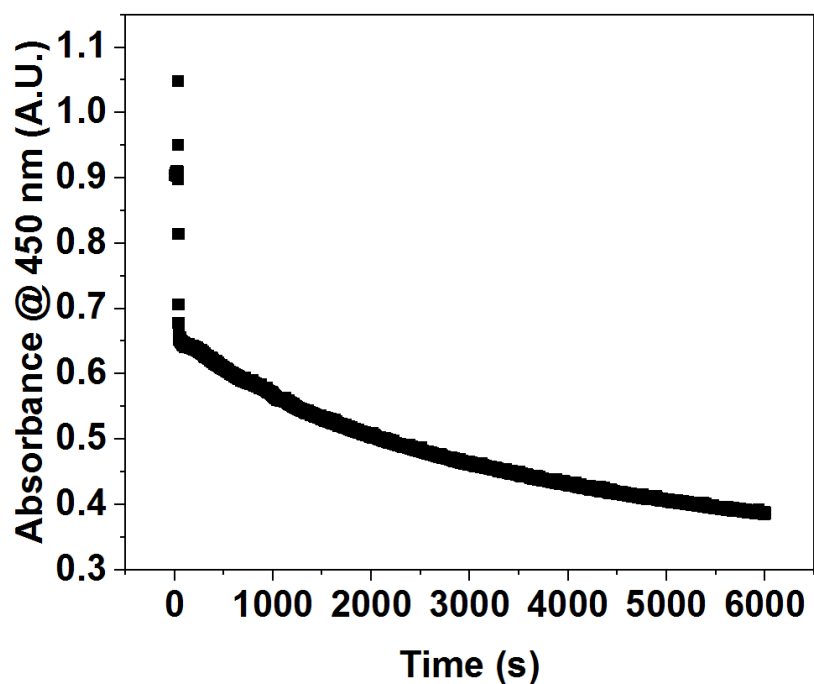


Figure A6.21: Change in absorbance at 450 nm over time during the reaction of **9** + 30 eq. TEMPO-H at -90 °C in 1:9 MeCN/THF.